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DIFFERENTIAL SECRETION OF ADRENALINE AND NORADRENALINE

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FOLLOWING THE DEMONSTRATION of the presence of noradrenaline in the adrenal medulla, the problem of its physiological meaning and of the noradrenaline content in the adrenal blood, arose.- At the present there are two concepts of its physiological significance. One is that noradrenaline is a chemical precursor in the synthesis of adrenaline (Blaschko, 1942; Bülbring, 1949) and the other, that the formation and secretion of noradrenaline is independent of that of adrenaline (Rapela and Houssay, 1952, c; Euler, 1954, a, b). During the last years, several investigators (Brauner and col. 1951; Brücke and col. 1952; Redgate and Gellhorn, 1953 and Folkow and Euler, 1954) have demonstrated that the stimulation of different parts of the hypothalamus changed the proportion of adrenaline to noradrenaline in the adrenal venous blood. Hillarp and Hokfelt (1953, 1954) and Eränko (1951, 1955) have described two types of adrenal medullary cells, one that may secrete adrenaline and the other noradrenaline.

Several stimuli, such as asphyxia (Tournade and Chabrol, 1924; Houssay and Molinelli, 1925 a; Malmejac and Chardon, 1947; Celander, 1954), nicotine injected intravenously (Houssay and Molinelli, 1925, b; Molinelli, 1926; van Slyke and Larson, 1950), electrical stimulation of the greater splanchnic nerve (Molinelli, 1926) are well known for their ability to induce the adreno-medullary cells to secrete.

A series of experiments were made to study the effect of these stimuli on the relative secretion of adrenaline and noradrenaline, by the adrenal gland.

It was found that the intravenous injection of nicotine (Rapela and Houssay, 1952, c) and the electrical stimulation of the greater splanchnic nerve (Rapela and Houssay, 1952, b; Rapela and Covián, 1954) induced preferentially the secretion of adrenaline or noradrenaline, while asphyxia

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(Rapela and Houssay, 1952, a) did not produce any change in the proportion of the adrenaline and noradrenaline secreted.

METHODS

Dogs with both vagi severed at the neck and with artifitial respiration were used. When the effect of asphyxia and of electrical stimulation of the splanchnic nerve by induced current was studied, the dogs were anesthetized with cloralose, 100 mg/kg body weight, injected intravenously. When the effect of nicotine and of stimulation of the splanchnic nerve by electrical current or pulses of known frequencies was studied, 33 mg/kg of nembutal was given intravenously.- Heparin was given intravenously in the dosage of 3 mg/kg, and then 1 mg/kg every 20 minutes. The adrenal venous blood was collected by cannulating the left lumbo-adrenal vein with a polyethylene tubing, so that all the blood from the adrenal would flow through the cannula, the adrenal vein between the cava and the adrenal gland was ligated. The blood was collected in graduated cylinders rinsed with a 1 % heparin solution, which were kept in an iced water bath.

The rate of blood flow was recorded. The plasma was separated by centrifugation at 4° C and then kept at 6° C for 2 to 3 hours, before being analized. The proportion between plasma and cells was determined with Wintrobe tubes.

The concentration of adrenaline and noradrenaline in the plasma was determined by comparing the equiefector doses of adrenaline in the rat uterus during diestrous (Jalon and col. 1945); Gaddum and Lembeck, 1949) and on the arterial blood pressure of the dog. The difference in sensitivity of both tests to adrenaline and to noradrenaline provide a method of determining the concentration of these substances (Gaddum and Lembeck, 1949).

The results are expressed as the ammounts of adrenaline and of noradrenaline, μg/kg body weight in the plasma that flowed from the adrenal in one minute.

Asphyxia. — Asphyxia was induced by occluding the trachea. The adrenal blood was collected from the 2nd to the 5th minute after occluding the trachea and 30 seconds after its release and the restoration of artificial respiration. The periods of asphyxia were repetted at 10 minutes intervals (Rapela and Houssay, 1952, a).

Nicotine injection. — Nicotine was injected intravenously in the safenous vein at the doses of 0.25, 0.50, 0.75, 1 and 1.5 mg. Adrenal blood was collected for 2 ½ minutes from the beginning of the injection. Subsequent blood collections and injections were made every ten minutes (Rapela and Houssay, 1952, c).

Section of the greater splanchnic nerve. — The major splanchnic nerve was divided inmediately below the diaphragm. The adrenal blood was collected after 15 minutes, 3-5 hours and 24 or 48 hours. Samples of adrenal blood were obtained previous to the section of the nerve in those experiments where adrenal blood was collected 15 minutes and 3-5 hours afterwards (Rapela and Houssay, 1952, b).

Electrical stimulation of the greater splanchnic nerve. — The effect

of the electrical stimulation of the greater splanchnic nerve was studied in two groups of experiments: a) when a Harvard inductorium was used the same voltage on the inductor and setting of the vibrator was mantained throughout each experiment, although they could have been different from one to another experiment (1); b) in another group of experiments the effect of the splanchnic nerve stimulation by monophasic rectangular pulses of 5 msec of duration or sinusoidal current, both of different frequencies and supramaximal intensity (3-6 volts), were studied. An electronic stimulator was used (Grass mod. 3 C). The sinusoidal current was taken from the oscilator circuit of the grass intercalating a cathode follower. Through a lumbar approach the greater splanchnic nerve was divided at the level of the ninth or tenth thoracic vertebra. In this way a segment of the nerve 5-6 cm, was freed and could be easily stimulated with a bipolar electrode. The stimuli were applied for a duration of 30 seconds each minute.

In all the experiments the stimuli were repeated until the amount of blood necessary for the determination of adrenaline and noradrenaline concentrations had been collected.

Results and discussion

Asphyxia. — The secretion of total catechols was increased about 40 times the value obtained in the controls, but there was no change in the proportion of adrenaline and noradrenaline secreted (Table I).

TABLE I

Adrenaline and noradrenaline in the adrenal venous blood. Asphyxia in the anesthetized dog

	Nº of Dogs	$\frac{\text{Adrenaline}}{\text{Noradr.} + \text{Adren.}} \times 100$	Adrenaline + Noradrenaline µg/min./kg.
Mean before asphyxia	10	86.8 ± 5.69	0.013 ± 0.003
Mean during asphyxia	6	85.6 ± 4.28	0.437 ± 0.141

The mechanism by which the secretion of total catechols is increased during asphyxia is complex. Besides the direct effect of anoxia on the adrenals (Bulbring, Burn and D'Elio, 1948) and an indirect effect through

(1) The characteristics of the current generated by the Harvard inductorium used in these experiments were analized subsequently with the osciloscope. According to the position of the vibrator the frequency changed from 18 to 70 cycles per second. The duration of the current generated during the closure (1/40 of the cycle) and opening of the primary circuit was wery short; during the latter the current generated on the secondary coil reached 40 volts at the peak of the wave when an output resistance of 2000 ohms was used.

the adrenaline secretory nervous centers, a reflex nervous action is predominant (Houssay and Molinelli, 1925, a; Malmejac and Chardon, 1947; Celander, 1954). The proportion of adrenaline to total catechols in the asphyxiated animals was similar to that of the controls, also dependent on the nervous factors. Since the ischemia in the denervated adrenal gland

TABLE II

Adrenaline and noradrenaline in the adrenal venous blood. Intravenous injection of nicotine (splanchnic nerve severed)

Exper. Nº	mg.	Adrenaline / Adren. + Noradr. × 100		Total catechols (Adren. + Noradren.) µg/min./kg.	
		Control	During Injection	Control	During Injection
10	0.50	75	93	0.014	0.402
	0.75		98		0.737
12	0.25	44	60	0.010	0.004
	0.50		78		0.034
14	0.50		96		0.187
	0.75	78	96	0.012	0.257
	1.50		98		0.507
15	0.50		33		0.012
	0.75	33	90	0.004	0.241
	1.50		89		0.277
16	0.50		82		0.240
	0.75	43	69	0.010	0.792
	1.50		86		1.856
17	0.75	85	94	0.010	0.277
	1.50		95		2.157

decreases the percentage of adrenaline secreted (Malmejac and col., 1952) while it does not change during asphyxia in the innervated gland, it would seem that the adrenaline percentage during asphyxia depends on the nervous action.

Nicotine. — In a study of the effect of nicotine on the proportion of adrenaline and noradrenaline secreted by the adrenal medulla, it was found that there was an increase in the percentage of adrenaline (Table II),

(Rapela and Houssay, 1952, c). These results have been confirmed in the cat by Folkow and Euler (1954). The preferential effect on the adrenaline secretion seems to be independent of the innervation of the gland. In the dog is best shown when the splanchnic nerve is cut.

The amount of total catechols, adrenaline and noradrenaline, increased markedly and proportionally with the increase in dosage. But, not clear correlation was found between dosage and the adrenaline percentage (Table II).

TABLE III

Adrenaline and noradrenaline in the adrenal venous blood. Stimuli of similar intensity (1) by nicotine injection and splanchnic electrical stimulation (Splanchnic nerve severed)

Increase in the proportion of $\frac{\text{Adrenaline}}{\text{Adren. + Noradren.}} \times 100$			Adren. + Noradren. µg/min./kg.	
Nicotine i. v. (8 exper.) 1	Splanchnic stimulation (17 exper.) 2	Difference between 1 and 2	Nicotine (8 exper.)	Splanchnic stimulation (17 exper.)
26 ± 5.54	10.3 ± 2.77	15.7	0.389	0.308
$P < 0.01$	$P < 0.01$	$P < 0.01$		

(1) Total catechol secretion (adrenaline + noradrenaline) is considered as an index of the intensity of the stimuli.

i. v.: intravenously; ± : Standard error; P: probability stimated by the "t" test.

Considering experiments in which the increase in the secretion of total catechols was similar, nicotine produced a preferential secretion of adrenaline greater than the splanchnic nerve stimulation (Table III).

The mechanism by which nicotine increases the secretion of adrenaline is not well known as yet. Though nicotine has a methyl group in its chemical structure it is not known wether it may have any effect as a methylating agent of the noradrenaline, probable precursor of the adrenaline (Blaschko, 1942; Bulbring, 1949). Another explanation could be that nicotine stimulates predominantly the adrenalinosecretory cells of the adrenal medulla.

Eränko (1955) obtained a similar finding in the adrenal medulla of the rat using histochemical methods. The injection of 1 mg per 100 g body weight of nicotine produced an increased concentration of adrenaline in the adrenal gland of rats killed four hours after the injection.

The changes in the adrenaline and noradrenaline content of the adrenal medulla after the chronic injection of nicotine have been studied

by West (1952), who found that the daily administration of nicotine by subcutaneous injection to rats and rabbits for periods up to 90 days, increased the proportion of noradrenaline. It was interpreted as a lowered rate of methylation, though no balances studies were made.

TABLE IV

Adrenaline and noradrenaline in the adrenal venous blood. Splanchnic nerve section

Exper. Nº	Adrenaline / Adren. + Noradren. × 100			Total catechols (Adren. + Noradren.) µg/min./kg.	
	$P1_1$ Control	$P1_2$ 2 min. up to 3-5 hr. after splanchnic section	$\Delta P1_2$-$P1_1$	Control	After splanchnic nerve section
N 14	100	78	—22	0.042	0.012
N 15	43	33	—10	0.006	0.004
N 16	55	43	—12	0.017	0.011
N 21	100	81	—19	0.071	0.004
E 7	100	62	—38	0.025	0.002
E 14	100	86	—14	0.007	0.008
E 17	70	48	—22	0.003	0.002
E 19	100	60	—40	0.005	0.004
E 20	100	43	—57	0.004	0.004
E 21	75	71	— 4	0.116	0.058
E 22	78	70	— 8	0.010	0.012
Mean	83.7	61.4	—22.3	0.028	0.011
S. E.			± 4.92		
P			< 0.01		

S. E.: Standard error; P: probability stimated by the "t" test.

Section of the greater splanchnic nerve. — From 2 minutes up to 3-5 hours after the section of the nerve there was a decrease in the total catechol secretion and a decrease in the adrenaline percentage (Table IV) (Rapela and Houssay, 1952, b).

The mean adrenaline percentage with the nerve intact was 83 % and after being divided 61 %. The difference between the means, 22 %, was statistically significant ($p < 0.01$). At 24-48 hours after the nerve

TABLE V

Adrenaline and noradrenaline in the adrenal venous blood. Splanchnic nerve stimulation with a Harvard inductorium

Time Between section and stimulation	Adrenaline / Adren. + Noradren. × 100				Rate of secretion of total catechols (Adren. + Noradren.) µg/kg. per min.)	
	Exper. Nº	Control (1) 1	Stimulated 2	Difference between 2-1	Control	Stimulated
	13	45	83	38	0.007	0.197
10-15 min.	15	55	73	18	0.002	0.541
	16	100	100	0	0.010	0.517
	7	62	83	21	0.002	0.234
	14	86	90	4	0.009	0.293
	16	100	100	0	0.020	0.452
3-5 hours	17	48	45	— 3	0.003	0.604
	19	60	82	22	0.005	0.301
	20	43	55	12	0.004	0.356
	21	71	88	17	0.059	0.984
	22	70	69	— 1	0.012	0.194
	9	67	77	10	0.010	0.209
	10	80	92	12	0.003	0.405
24-48 hours	12	64	73	9	0.007	0.309
	13	76	91	15	0.005	0.123
	15	63	63	0	0.003	0.469
	18	89	87	— 2	0.004	0.436
Mean				10.3	0.010	0.390
Standard error				± 2.77		
P				< 0.01		

(1) Samples taken after the nerve was severed.
P: probability estimated by tne "t" test.

was cut the mean of the adrenaline percentage was 73 %. Though this value is lower than the one observed with the nerve intact (8.4 ± 4) in other experiments, the difference was statistically no significant. Because of technical difficulties control values in the same animals could not be obtained.

Splanchnic nerve electrical stimulation.

a) *Induced current.* — When the greater splanchnic nerve was stimulated with a Harvard inductorium (Rapela and Houssay, 1952, b) an increased in the percentage of adrenaline in the adrenal blood was observed and, as it is well known, there was also a marked increase in the amount of total catechols (adrenaline + noradrenaline) secreted.

Table V shows the effect of stimulating the splanchnic nerve with an inductorium at 10-15 min. 3-5 hours and 24-48 hours after cutting the nerve. The secretion of total catechols was increased 39 times. The average increase in the adrenaline percentage was 10.3 % ($p < 0.01$). This increase was observed in 11 experiments; in 3, the percentage of adrenaline was unchanged and in the other 3 a slight decrease was found after the stimulation of the nerve. The time elapsed between the section of the nerve and the stimulation apparently did not influenced the changes observed in the adrenaline percentage.

These results agree with those obtained by other authors. Bulbring, Burn and D'Elio (1948) found an increase in the synthesis of total catechols, in the perfused adrenal gland of the dog when the greater splanchnic nerve was stimulated. Bulbring (1949) in "in vitro" studies of the methylation of noradrenaline when minced adrenal gland was added, and Malmejac and col. (1952, 1953) in experiments with an adreno-yugular anastomosis and adrenergic blocking agents observed, respectively, a greater adrenaline formation and an increased percentage of adrenaline in the adrenal venous blood, when the greater splanchnic nerve was stimulated. Holtz and col. (1952) stimulating the splanchnic nerve of the cat found that the adrenal venous blood had nearly only adrenaline.

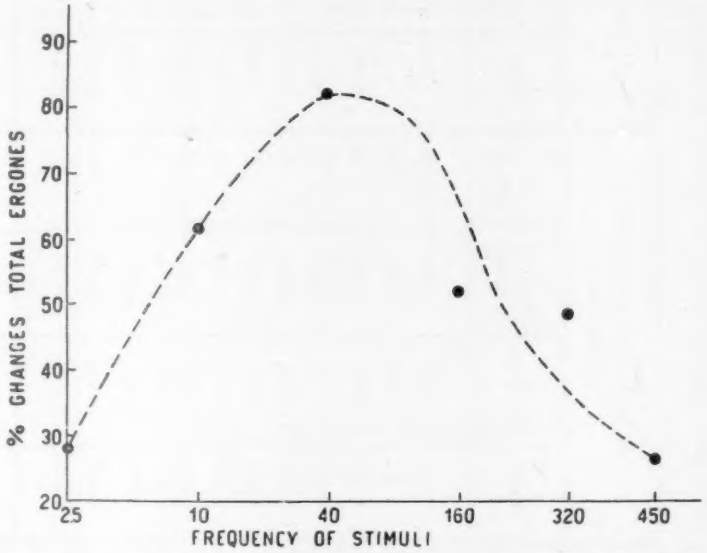
The results obtained with the section and stimulation of the greater splanchnic nerve show that the latter, in addition to its known tonic effect on the secretion of total catechols of the adrenal medulla, has an effect on the proportion of adrenaline and noradrenaline secreted in the adrenal venous blood.

b) *Electrical stimuli of different frequencies.*

1º) *Effect on total catechol secretion.* — The adrenal venous blood was tested on the blood pressure of the dog and meassured as equieffective doses of adrenaline. Supramaximal stimuli of different frequencies induced the adrenal medulla to secret according to a curve with a maximun around 40 stimuli per second decreasing with higher frequencies (graph. 1) (Rapela and Covián, 1954). In some experiments this maximum was observed with 10 stimuli per second or exceptionally with up to 160 per second. The curve obtained was similar when sinusoidal current or rectangular pulses of similar frequencies were applied.

The amount of current per unit time applied to the nerve did not influence the total amount of catechols obtained with rectangular pulses. In one experiment, when the duration of the pulses was changed in relation to its frequency so that the amount of current per second were the same, similar responses were obtained as when stimuli of different frequencies and the same duration were applied. These results could be expected considering that the nerve response follows the all or none law. According to the neuro humoral theory of the transmission of the nerve impulse,

which can be applied to the mechanism of the secretion of the adrenal medulla (Feldberg and col., 1934), each supramaximal impulse should produce the same amount of chemical transmitter, the total amount secreted depending on the frequency of the stimuli, but not on the amount of current. As a consequence, the increase in secretion with pulses up to 40 per second has been explained as the result of a temporal summation of the stimuli (Houssay and Molinelli, 1928; Rosenblueth, 1932; Celander, 1954).



GRAPH. 1.—*Total catechol secretion of the adrenal gland during the electrical stimulation of the splanchnic nerve.* Abscisae: *Pulses of different frequencies, supramaximal voltages and 5 msec duration.* Ordinates: *Mean of the percental changes, respect to the maximum secretion in each experiment, observed in 14 experiments. Total catechol secretion measured as equieffector doses of adrenaline in the blood pressure of the dog.*

The decrease in the response with frequencies higher than 40-50 stimuli per second could depend on the incidence of the succesive impulses within the refractory period (Houssay and Molinelli, 1928; Rosenblueth, 1932). It is assumed that the refractory period of the neuroglandular synapse in the adrenal medulla is the same to that in the cervical sympathetic ganglion, which is 20 msec. (Bishop and Heinbecker, 1930).

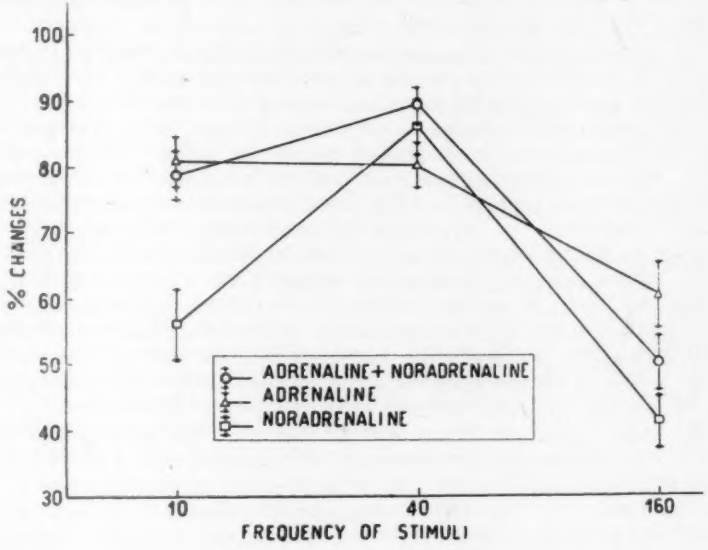
Celander (1954), found that in general the maximal secretion was obtained with frequencies ranging from 10 to 20 stimuli per second. He used a method equally sensitive to noradrenaline and adrenaline, whereas we used a method more sensitive to noradrenaline. Since there is increase in the proportion of noradrenaline to adrenaline with a frequency of stimulation of 40 pulses per second (see below) the use of the latter method will give an apparent increase in the total catechols.

TABLE VI

Adrenaline and noradrenaline in the adrenal venous blood
Stimulation of the splanchnic nerve with electrical pulses of different fraquencies

Exper. Nº	Body weight kg.	10 pulses/sec. — 5 msec. µg/min./kg.				40 pulses/sec. — 5 msec. µg/min./kg.				160 pulses/sec. — 5 msec. µg/min./kg.			
		Adren.	Noradren.	Adren. + Noradren.	% Adren.	Adren.	Noradren.	Adren. + Noradren.	% Adren.	Adren.	Noradren.	Adren. + Noradren.	% Adren.
12	9.2	0.136	0.051	0.187	73	0.134	0.138	0.272	49	0.063	0.037	0.100	63
13	8.2	0.079	—	0.079	100	0.233	0.041	0.274	85	—	—	—	—
16	16	0.164	0.033	0.197	83	0.196	0.089	0.285	69	0.154	0.033	0.187	82
17	11.5	0.235	0.209	0.444	53	0.496	—	0.496	100	0.261	—	0.261	100
21	15	0.214	—	0.214	100	0.128	0.106	0.234	55	0.009	0.006	0.015	60
22	13	0.254	0.042	0.296	86	0.283	0.117	0.400	71	0.300	0.059	0.359	84
23	15	0.168	0.034	0.202	83	0.190	0.152	0.342	55	0.521	0.194	0.715	73
25	15	0.113	0.028	0.141	80	0.129	0.025	0.154	84	0.035	0.018	0.053	66
26	13	0.190	0.023	0.213	89	0.138	0.035	0.173	80	0.084	0.014	0.098	86
27	12	0.071	0.046	0.117	61	0.031	0.033	0.064	48	0.024	0.020	0.044	55
28	10	0.125	0.067	0.192	65	0.102	0.080	0.182	56	0.117	0.035	0.152	77
29	10.5	0.065	0.125	0.190	34	0.049	0.130	0.179	27	0.054	0.041	0.095	57
Mean		0.151	0.054	0.205	75.6	0.175	0.079	0.254	64.9	0.147	0.042	0.189	73

2º) *Effect on adrenaline and noradrenaline secretion.* — When the adrenal venous plasma were tested to differentiate adrenaline and noradrenaline it was found in 10 out of 12 experiments, that the amount and the proportion of noradrenaline secreted were greater when the frequency of the stimulus was 40 pulses per second than when 10 or 160 per second (Table VI). In order to evoid a systematic error due to changes of the condition of the animal during the experiment, a random order of the frequencies of the stimuli applied was followed. It was observed in several experiments, that the effect of the stimuli of different frequencies



GRAPH 2. — *Adrenaline, noradrenaline and adrenaline + noradrenaline secretion during the electrical stimulation of the splanchnic nerve.* Abscisae: *Pulses of different frequencies, supramaximal voltages and 5 msec duration.* Ordinates: *Mean of the percental changes, respect to the maximum secretion in each experiment, observed in 12 experiments.*

on the amount and proportion of noradrenaline secreted was independent of the order of applying the different stimuli.

The maximum of the noradrenaline secretion with a stimulus of 40 pulses per second is better shown if the percental changes with the different frequencies are considered. Graph 2 shows the mean of the percental changes in respect to the maximum value in each experiment, for the noradrenaline, adrenaline and total catechols secretion when stimulating the splanchnic nerve with 10, 40 and 160 pulses per second. There was a significant difference in the noradrenaline secretion between stimuli of 10 and 40 pulses per second ($P < 0.05$) whereas no difference in the adrenaline and total catechols, was observed.

The changes observed with 160 pulses per second are of interest in that the succesive impulses would probably occur within the absolute or relative refractory period. The proportion of adrenaline in the adrenal venous blood was greater when the nerve was stimulated with 160 pulses per second than with 40 per second.

General discussion

The demonstration of noradrenaline in the adrenal medulla and in the adrenal venous blood raises the problem of whether noradrenaline is just a chemical precursor of adrenaline or that it is secreted independently and has a definite physiological role.

The results obtained in our experiments suggest that a preferential secretion of noradrenaline and of adrenaline may exist. The analisis of the adrenal venous plasma obtained during stimulation of the greater splanchnic nerve with electrical stimuli of 10 and 40 pulses per second shows that noradrenaline increased when 40 pulses per second were applied, whereas there was a relative small change in the amount of adrenaline secreted (graph. 2, table VI). The obvious consequence of this is that the proportion of noradrenaline increases. The relatively small difference in the secretion of total catechols when stimulating with 40 or with 10 pulses per second does not support the interpretation that the increased secretion of noradrenaline results of an unbalanced formation of adrenaline respect to its secretion, but rather of a preferential secretion of noradrenaline. Gaddum and Lembeck (1949) did not find any evidence of a loss of adrenal's power of methylation even when the splanchnic nerve of the cat was continuously stimulated over 45 minutes.

The tonic action of the splanchnic nerve on the secretion of adrenal medullary hormones and the electrical stimulation with a Harvard inductorium influenced differently the proportion of adrenaline and noradrenaline secreted, than the stimulation with 40 pulses per second. The severance of the greater splanchnic nerve (Rapela and Houssay, 1952, b) produced a decrease (table IV) and its stimulation with a Harvard inductorium an increase (table V) of the proportion of adrenaline in the adrenal venous blood.

Among other stimuli inducing a preferential secretion of adrenaline, the intravenous injection of nicotine has a marked effect (table II). (Rapela and Houssay, 1952, c).

The preferential secretion of adrenaline in the adrenal blood of the cat has also been shown by other authors, stimulating the secretion by different ways: the stimulation of different points of the hypothalamus (Brauner and col., 1951; Brücke and col., 1952; Redgate and Gellhorn 1953; Folkow and Euler, 1954) induced the secretion of different proportions of adrenaline; the hyperglycemia induced by the intravenous injection of glucose (Duner, 1954) provoked a decreased secretion of adrenaline without major changes in the noradrenaline; the stimulation of the sciatic nerve determines a reflex predominant liberation of adrenaline (Euler y Folkow, 1953).

Other stimuli as the asphyxia (Table I) though induces a marked

increase of the catechols secretion does not change the proportion of the adrenaline and noradrenaline in the adrenal blood.

The facts so far observed show that stimuli mediated through the splanchnic nerve as the stimulation of the splanchnic major nerve with electrical pulses of different frequencies, asphyxia, electrical stimulation of the hypothalamus, hyperglycemia, stimulation of the sciatic nerve, or direct stimuli of secretory cells as after the intravenous injection of nicotine induce a preferential secretion of one or the other of the adrenomedullary hormones.

The simplest mechanism through which this differential secretion could be acomplished would result of the existence of sympathetic nervous fibers, not as yet demonstrated, that would be excited preferentially by certain stimuli and would end in adrenal medullary cells that secret preferentially or exclusively noradrenaline or adrenaline (Hillarp and Hokfelt, 1953, 1954; Eränko, 1951, 1955). In regard to this hypothesis the experiments of Folkow and Euler (1954) suggest the existence of areas in the hypothalamus which, when stimulated, induce a specific secretion of adrenaline or noradrenaline (Euler, 1954, a, b).

The effect of the allready mentioned stimuli on the rate of noradrenaline sinthesis and its methylation to adrenaline and on the rate of adrenaline secretion is another alternative explanation that cannot be disregarded as yet. Though many experimental facts show the possibility that noradrenaline be a chemical precursor of adrenaline (Blaschko, 1942; Bulbring, 1949) the demonstration by Hillarp and Hökfelt (1953, 1954) and Eränko (1951, 1955) of the existence of adrenal medullary cells especifically diferentiated for the formation of adrenaline or noradrenaline constitute a strong support to the former hypothesis.

Summary

The effect of different stimuli on the adrenal medullary secretion and its proportion of adrenaline and noradrenaline have been studied in the adrenal venous blood of the dog using a biological test.

Asphyxia increased 40 times the adrenal medullary secretion, with no changes in the proportion of adrenaline and noradrenaline.

The section of the greater splanchnic nerve decreased the adrenal medullary secretion and the proportion of adrenaline (—22 %). Its stimulation by a Harvard inductorium increased 39 times the total catechol secretion and the proportion of adrenaline secreted (+ 10 %).

The electrical stimulation of the greater splanchnic nerve by square pulses of different frequencies and supramaximal voltages induced a greater proportion of noradrenaline with 40 pulses per second than with 10 o 160 per second.

The intravenous injection of nicotine (0.25-1.5 mg) determined a marked increase of the total catechol secretion and of the proportion of adrenaline.

The meaning of these results in relation with the hypothesis of a preferential secretion of adrenaline or noradrenaline by the adrenal medulla is discussed.

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DESCRIPTION OF THE CIRCULATORY DYNAMICS IN THE HEART AND LUNGS IN MITRAL STENOSIS BY MEANS OF THE DYE DILUTION TECHNIQUE

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THE DYE DILUTION technique using T-1824 (Evans blue) is widely known. In a single experiment the circulation time, cardiac output, intrathoracic blood volume (Hamilton), central volume (Newman) and total blood volume may be measured. In a previous paper ([14]) we have mentioned the fundamental references and discussed some theoretical aspects.

Anatomopathological ([2], [10], [16]) and hemodynamic studies (using cardiac catheterization) ([12], [13], [18], [19]) have shown that in mitral stenosis the lumen of the arterioles is reduced. This suggested a reduction in pulmonary blood volume. Nevertheless, a concomitant dilatation and engorgement of the pulmonary capillaries has been described which would counteract the arteriolar constriction. Since the pulmonary capillary bed represents only 10 per cent of the blood volume (Roughton, [17]), the possibility that the dilatation of the lung capillaries would not lead to any considerable increase of the pulmonary blood volume was considered.

The pulmonary blood volume was measured by means of the dilution curve of Evans blue in 20 patients with mitral stenosis of various degrees. Other hemodynamic values obtainable from the dilution curve were calculated.

MATERIAL AND METHODS

Twenty patients with typical mitral stenosis were studied. The selection criteria used was essentially clinical, as recomended by Janton et al. ([7]) for the determination of mitral stenosis and by Dexter ([3]) for the elimination of other valvular diseases. The age of the patients varied from 15 to 33 years; 4 were males and the rest females. The patients were grouped in grades according to Dexter et al. ([12]). No patients were of grade I, 7 corresponded to grade II, 6 to grade III and 7 to grade IV.

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The diagnosis was confirmed in 6 patients (Nos. 3, 4, 8, 13, 14 and 17) during operation; none of them had mitral insufficiency.

All patients were studied in basal conditions after a rest of at least 45 min. The method and calculations used have been previously described ([14]).

RESULTS

Our results corrected per m^2 of body surface are given in Table I.

We have previously stated ([14]) that a significant sexual difference exists only in blood volume and intrathoracic volume; for this reason when calculating the mean of these values only data obtained in women (the more numerous) were considered, comparing them with the values obtained in normal women. For all the other values sex was disregarded.

Fig. 1 shows our normal values at sea level ([14]) compared with those obtained in mitral stenosis (mean $\pm$ one standard deviation); they have been plotted in semi-logarithmic paper in order to facilitate comparison of the different values.

Circulation times. — The appearance time and the mean circulation time increase in a parallel manner with the severity of the disease. This increase is statistically significant ($p < 0.01$) in our patients of grade II as compared with normals. No statistical significant difference was found between means of grades II and III, but in grade IV the mean was significantly greater than in the other groups ($p < 0.01$).

The build-up time was greater the greater the degree of stenosis, the difference of means of succesive groups being significant (between normals and grade II, $p < 0.01$; between grades II and III, $p = 0.03$; between grades III and IV, $p < 0.01$).

Cardiac index. — The cardiac index was reduced in a parallel manner with the severity of the disease. This reduction was significant in grade II when compared to normals ($p < 0.01$). No difference was observed between grades II and III; but in patients of grade IV the cardiac index was significantly lower than in the preceding groups ($p < 0.01$).

Central volume. — (Newman, [15]). Values of central volume in patients of grade II did not differ from normal. In patients of grade III they were significantly greater than in the preceding ($p < 0.01$). No difference existed between values of grades III and IV.

Intrathoracic blood volume. — (Hamilton, [16]). Although the values tended to increase with the severity of the disease, differences between means were not statistically significant.

Blood volume. — The values increased in a parallel manner with the severity of the disease. The difference between normal values and those of grade II was not significant; but there is a significant difference between those of grade III and the preceding groups ($p < 0.01$). In grade IV the mean is greater than in grade III the difference being in the limit of statistical significance ($p = 0.057$).

Central volume expressed as percentage of blood volume. — No significant difference existed between grades II and IV with respect to normals. In group III the mean was significantly greater ($p = 0.035$).

Intrathoracic blood volume expressed as percentage of blood volume. No significant difference existed between the means of groups II and III

TABLE I

	SEX	AGE	G	W	SA	AT	BUT	MCT	Ht	CI	CV	ITV	TBV	$\frac{CV}{TBV} \times 100$	$\frac{ITV}{TBV} \times 100$
				Kg.	m^2	s/m^2	s/m^2	s/m^2	%	$L/m/m^2$	L/m^2	L/m^2	L/m^2	%	%
1	M	15	II	49.5	1.49	9.9	5.7	23.0	39	3.20	0.38	1.83	2.50	15	73
2	M	24	II	62.0	1.65	12.5	6,2	21.6	45	2.86	0.47	1.70	2.44	19	70
3	M	36	II	60.0	1.62	13.8	6.2	24.3	50	2.32	0.43	1.52	3.18	14	48
4	F	21	II	46.7	1.41	14.0	6.2	22.4	39	3.00	0.42	1.58	3.36	12	47
5	F	53	II	73.5	1.76	14.0	9.6	26.2	41	1.25	0.22	0.96	1.90	11	51
6	F	21	II	43.5	1.34	8.9	7.4	17.9	37	3.12	0.36	1.24	1.87	19	67
7	F	38	II	55.3	1.50	12.1	10.6	25.3	45	2.07	0.38	1.31	2.34	16	56
8	F	33	III	42.0	1.31	10.7	9.6	23.5	37	3.94	0.55	2.03	3.12	18	65
9	F	22	III	50.0	1.50	8.8	8.6	19.9	42	2.35	0.42	1.17	2.70	16	43
10	F	38	III	58.9	1.62	11.5	8.7	26.8	47	2.24	0.77	1.62	3.06	25	53
11	F	31	III	40.6	1.29	11.5	8.2	23.3	32	3.06	0.56	1.53	3.06	18	50
12	F	29	III	55.0	1.63	16.3	11.3	31.4	43	2.08	0.64	1.77	2.98	22	59
13	F	25	III	42.3	1.27	14.2	7.7	28.6	40	3.07	0.70	1.86	2.37	29	74
14	M	44	IV	50.5	1.51	21.6	14.3	40.0	43	1.51	0.78	1.52	3.45	23	44
15	F	37	IV	49.3	1.45	10.8	10.2	23.8	40	1.74	0.41	1.66	3.30	12	50
16	F	46	IV	46.0	1.34	17.7	8.9	41.8	43	1.87	0.81	1.74	3.74	22	47
17	F	31	IV	43.1	1.30	18.3	11.2	37.7	36	1.77	0.50	1.45	2.24	22	65

TABLE I (Cont.)

	SEX	AGE	G	W	SA	AT	BUT	MCT	Ht.	CI	CV	ITV	TBV	$\frac{CV}{TBV}$ x 100	$\frac{ITV}{TBV}$ x 100
				Kg.	m^2	s/m^2	s/m^2	s/m^2	%	L/ m/m^2	L/m^2	L/m^2	L/m^2	%	%
18	F	43	IV	57.2	1.56	19.2	12.8	36.8	44	1.73	0.51	1.67	3.90	13	43
19	F	36	IV	59.0	1.59	13.8	10.9	30.0	38	1.59	0.49	1.27	3.51	14	36
20	F	16	IV	38.0	1.27	15.8	13.8	42.8	37	2.10	0.64	1.89	3.30	19	57
M			II			12.2	7.4	23.0	40.5°	2.54	0.39	1.27°	2.37°	15.2	59
SD			II			2.0	1.3	2.7		0.71	0.08			3.1	11
M			III			12.2	9.0	25.6	40.5°	2.79	0.60	1.66	2.88	21	57
SD			III			2.6	1.3	4.1		0.70	0.12	0.32	0.29	5	11
M			IV			16.7	11.6	36.2	39.5°	1.76	0.59	1.61°	0.33°	17.9	49
SD			IV			3.6	1.5	6.9		0.35	0.15	0.22	0.54	4.3	9.6

W: Weight

SA: Surface area

AT: Appearance time

BUT: Build-up time

Ht.: Hematocrit

CI: Cardiac Index

CV: Central volume

ITV: Intrathoracic volume

TBV: Total blood volume

M: Mean

SD: Standard deviation

°: Only women are considered in the mean

when compared to normals. In group IV the mean was somewhat lower, the difference being in the limits of statistical significance ($p = 0.045$).

Discussion

Since previous authors using the dye dilution curve did not group the patients in grades and since the techniques used differed somewhat

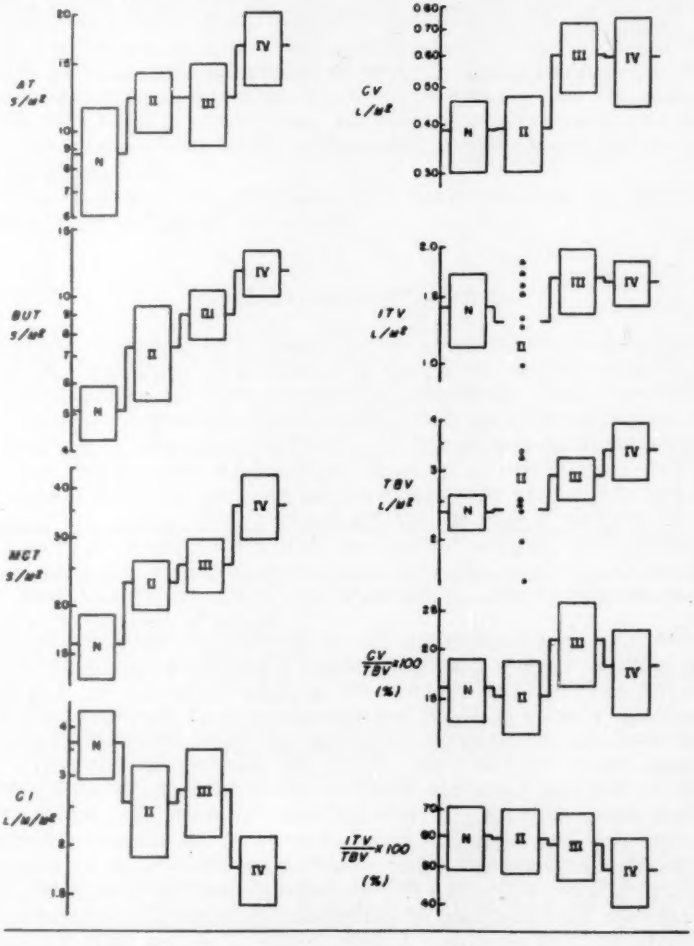

FIG. 1. — *Mean and standard deviation of our normal values at sea level (N) compared to those in mitral stenosis of grades II, III and IV. For explanation of symbols see Table I, ordinates in logarithmic scale.*

from ours, the comparison of our values with those reported in the literature should be made with reservations.

Circulation times. — Newman [15] in his glass models indicates that the build-up time is governed by the minor volumes simulating the cardiac chambers. If this applies to human beings one could speculate that the prolongation in the build-up times in our patients indicates an increase in size of the heart chambers.

Cardiac index. — Our findings are coincident with those reported and discussed in the literature using the dilution method [1, 5, 6] or the Fick principle [12, 13, 18, 19].

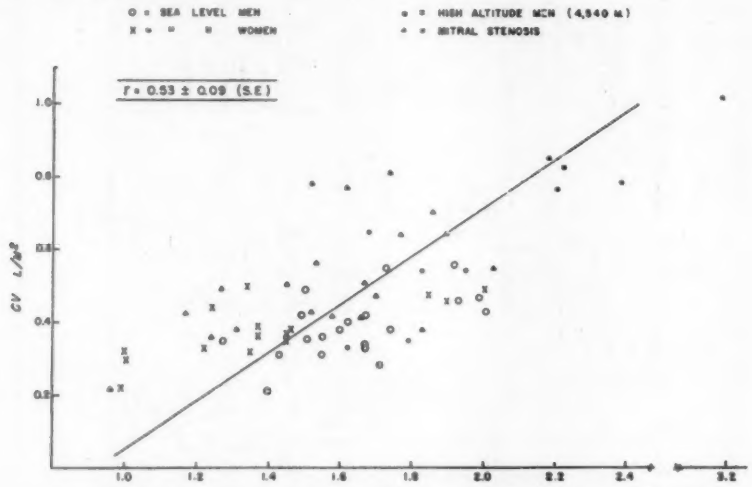

FIG. 2. — *Correlation between central volume (pulmonary blood volume, Newman) and intrathoracic volume (Hamilton).*

Pulmonary blood volume. — Its determination has been the subject of many studies, first by direct methods [4] and later by indirect methods. Two formula are used for its calculation: the one devised by Hamilton [6] by which the volume of blood between the site of injection and the site of blood sampling is calculated, including the blood contained in the lungs, heart and main vessels; the other by Newman's formula. If the conclusions derived from his study in glass models [15] and confirmed in experimental animals [11] is applicable to man, the central volume would represent the volume of blood existing in the lungs, with exclusion of the heart chambers and other vascular spaces.

In a previous publication [14] we have shown that a good correlation exists between both methods of calculation in normal subjets at sea level and natives at high altitude. A new figure is shown (fig. 2) in which results obtained in our mitral stenosis patients have been added. The correlation is statistically significant ($r \pm$ st. error $= 0.53 \pm 0.09$).

In most cases in the literature Hamilton's formula is used, but the injection of the dye is made in the pulmonary artery. Our injections were

made in the antecubital vein; our values being thus not strictly comparable. Our results coincide with those of Borden (1) and Kopelman (9) showing a slight non significant increase in mitral stenosis. Doyle (5) found an increase of 23 to 28 per cent of the intrathoracic volume expressed as percentage of the total blood volume.

Kattus (8) used Newman's formula. His normal mean is 0.59 $1/m^2$ and his mean in seven mitral patients is 0.49 $1/m^2$. Two of the seven patients, submitted to mitral commisurotomy, were studied post-operatively; five of the patients pertained to grade II and two to grade III. Since changes in total blood volume exist in mitral stenosis, it is convenient to express the pulmonary blood volumes as percentage of total blood volume and not in absolute values. Our results show a normal percentage in grades II and IV and an increase of 15 to 21 per cent in grade III.

This revision of the data from the literature and its comparison with our data seem to corroborate the absence of any systematic increase in the percentage of the blood volume contained in the lungs in patients with mitral stenosis.

Blood volume. — In our cases the blood volume, as known, was increased when compared to normal.

Summary and conclusions

A study was made in 20 cases of typical mitral stenosis of grades II, III and IV (according to Dexter) by means of the dye dilution curve. The following aspects were considered: circulation times, cardiac index, central volume (pulmonary blood volume, Newman) intrathoracic volume (Hamilton) and total blood volume, and the values obtained were compared with the normal values obtained by us at sea level.

Together with the severity of the disease the circulation times were prolonged, the cardiac index decreased and the blood volume increased.

Expressed as absolute values the central volume (pulmonary blood volume) increased in a parallel manner with the severity of the disease; but expressed as percentage of the blood volume this increase was significant only in patients of grade III.

The intrathoracic volume showed no significant variations.

A statistically significant correlation was found between central volume and intrathoracic volume.

Our results show that no systematic increase of the percentage of blood contained in the lungs occurs in mitral stenosis.

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THE EFFECT OF ADAPTATION TO CO ATMOSPHERES ON THE RATE OF BURNING OF CO BY FROG MUSCLE

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IT WAS SHOWN in 1932 Fenn and Cobb (1) that CO is burned to CO_2 by frog muscle. This was confirmed later by the use of radioactive carbon (2). The present report summarizes experiments carried out in 1948 in order to discover whether previous accommodation of the frogs to an atmosphere containing sub-lethal concentrations of CO would possibly facilitate or in any way modify the ability of muscle tissue to burn CO. The results, although not entirely conclusive, seem worthy of publication as a guide to future investigations in this field. The data obtained appear to indicate not an improvement in the burning but rather no change or an actual loss in the ability to burn CO after some weeks of accomodation to CO.

METHODS

The respirometer technique previously described (2) was used for most of the measurements with a few determinations by the isotope method. Using differential volumeters we measured the rate of oxygen consumption of an isolated frog muscle (sartorius) first in air, then in a mixture of 79 % CO and 21 % 0_2, and finally in air again. All muscles were left over night at 4° C in Ringers solution before the experiment. While in the respirometer they were suspended in 2 ml of Ringers solution at 22° C. The frogs were kept in a 50 liter tank at room temperature. CO was added to the tank air in increasing amounts so that after 3 weeks it reached 2 or 2.5 % starting with 0.1 %. A few frogs died during this process but the same number might have died without the CO. In any event, the concentration of CO was about as high at all times as the frogs could tolerate.

RESULTS

The results of most of the experiments by the respirometer technique are summarized in Table I. A few experiments were done with skin and

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nerve but only the heart and skeletal muscle data are sufficiently comprehensive to report. Two groups of frogs were used; Group 1 completed during August and September consisted of rather thin, starved frogs, and Group 2 in October and November represented another shipment of

TABLE I

The Rate of Burning of CO to CO_2 by Frog Muscle after Various Durations of Exposure to Atmospheres Containing CO

Treatment	Days	Nº of Expts.	Gas Uptake			% Increase
			Air	$O_2 + CO$	Air	
			cu. mm. /gm. min.			
Skeletal Muscle						
Control # 1	—	18	.40	.76	.41	87
CO	7	2	.40	.80	.34	116
CO	14-17	4	.42	.40	.44	–7
Control # 2	—	13	.42	1.31	.49	188
CO	7	3	.28	.44	.29	55
CO	17-24	12	.63	.42	.11	13
CO 20 days, then air	10	3	.40	.36	.28	6
CO 20 days, then air	20	2	.54	.70	.19	92
Air	14	5	.62	.68	.18	70
Air	21	5	.41	.80	.17	175
Air	28	12	.53	1.21	.48	140
Cardiac Muscle						
Control # 1	—	9	1.13	1.63	.65	84
CO	14-17	3	.96	1.38	.94	45
Control # 2	—	4	1.87	4.39	1.44	166
CO	7	1	.59	1.30	.54	130
CO	17-20	3	2.30	1.58	.86	0
CO 20 days, then air	10-20	2	1.10	1.08	.56	30
Air	14-21	3	1.88	2.36	.44	104
Air	28	3	1.86	4.02	1.38	148

animals in somewhat better condition. The general results were the same with both groups. Measurements of muscles from CO-acclimatized frogs were interspersed with measurements on normal frogs kept in air. In the first control group were 18 frogs with rates of O_2 consumption in air before and after CO of 0.40 and 0.41 cu.mm. per gm. per minute and a

rate in the CO-O_2 mixture of 0.76 which represented an 87 % increase over the rate in air. For comparison we have figures on 6 acclimatized frogs. Two of these tested after 7 days in CO burned CO in approximately normal amounts; of four others tested after 14-17 days in CO (CO=1.0 %) two were apparently normal but two showed an actual decrease in rate in the CO mixture. On the average there was a 7 % decrease in rate in the CO mixture.

In the second group of frogs there was a 183 % increase in rate due to CO in the controls and a decrease in rate of CO burning after 3 weeks of acclimatization in air containing 1.7 % CO. After 20 days of

TABLE II

The Effect of Exposure for 10 Hours to 1 Ahmosphere of CO on the Ability of Frog Muscles to Burn CO to CO_2

Treatment	Nº of Expts.	Gas Uptake			% Increase
		Air	O_2 + CO	Air	
		cu. mm. /gm. min.			
Skeletal Muscle					
1 atm. CO + 2 atm. O_2	23	.37	1.48	.42	278
1 atm. N_2 + 2 atm. O_2	19	.40	1.40	.40	249
Heart					
1 atm. CO + 2 atm. O_2	6	1.55	4.26	1.26	203
1 atm. N_2 + 2 atm. O_2	5	1.75	3.53	1.28	132

recovery in air the CO burning was still below normal but after 20 days of recovery it was more nearly normal in 3 frogs. For comparison 22 frogs were tested which had been kept in a similar 50 liter tank without CO and all of these gave approximately normal values for CO burning.

Experiments with cardiac muscle were qualitatively similar to those with skeletal muscle. After 14-17 days of acclimatization in the first group and 17-20 days in the second group the rate of CO burning was definitely diminished or absent (3 frogs in each case). There was some evidence of recovery when frogs were taken out of the CO and allowed to recover for 10-20 days. Frogs kept under similar conditions in air showed no decrease in CO burning.

Another somewhat different series of experiments is summarized in Table II. In this case frogs were exposed in a steel pressure chamber for 10 hours to 1 atmosphere of CO plus 2 atmospheres of O_2. The muscles were then dissected and left in Ringers solution at 4°C over night and were tested in the respirometers thereafter in the usual way. Control frogs were treated similarly except that the CO was replaced by N_2. Both cardiac and skeletal muscles were used. In both types of muscle the average percent increase is slightly larger in the CO-treated frogs but these differences are not statistically significant in either case. Brief

treatment with 1 atmosphere of CO therefore has no obvious effect on the capacity to burn CO.

Additional data were obtained by the isotope method. In this case the muscles were exposed for 5 hours to 80 % CO labelled with C^{14}. The CO_2 formed during this period was collected as $BaCO_3$ and the radioactivity of the C was determined as previously described (2). The amount of CO burned was calculated on the assumption that it was burned to CO_2. No significant differences were observed between the control muscles and those from frogs acclimatized for 23 days to CO (increasing in concentration up to 2.5 %). The amounts of CO burned (cu.mm.per gm. muscle per min.) were for skeletal muscles: controls 0.10, 0.11, 0.17, and 0.36, and for the acclimatized, 0.10, 0.04, 0.26, and 0.25. For heart muscle the figures were: for the controls, 0.56, 0.76, 0.63, and 0.65, and for the acclimatized, 0.56 and 0.59. We are indebted to Dr. R. T. Clarke, Jr., for these figures and the control values were those previously reported. (Clark et al, 1948, Table I).

Discussion

From these figures it must be concluded that there is no increase in the ability of frog muscles to burn CO after rather prolonged exposure to gradually increasing concentrations of CO in the atmosphere. The final concentration reached was large enough to be fatal if applied inmediately to an unacclimatized frog. For an adequate statistical treatment of the data there should have been more determinations on acclimatized frogs but the only trend which appeared was a regular decrease in the amount of CO burning after acclimatization. In some respects the data here are difficult to interpret because muscles from these acclimatized frogs seemed to show a falling base line, i.e., the rate of oxygen consumption in air after the CO-0_2 period tended to be less than the rate in air before this treatment, as shown in Table I. For these reasons we are not completely convinced that the ability to burn CO is diminished by acclimatization, especially since the direct determination of the CO burned by the isotope method did not show any trend in this direction. Further work is therefore necessary to demonstrate the point with certainty, but it seems certain that acclimatization is not accompanied by an increased ability to burn CO.

Summary

Exposure of frogs to sublethal concentrations of CO for periods up to 3 weeks did not increase and may possibly have decreased the ability of the heart and skeletal muscles to burn CO to CO_2.

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EXCRETION OF URINARY ESTROGENS IN THE PREGNANT BUFFALO

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THE ROLE OF ESTROGENS in the animal body is linked with many processes in fertility and sterility. The excretion of estrogens is a zoological phenomenon controlled by complex factors such as the species, age, reproductive phase, stage of pregnancy and the endocrine system. It is well established that estrogens are excreted in the various body fluids and mainly in the urine. Recent investigations have been carried out so as to show the forms, levels and physiological activities of estrogens in rodents and farm animals. It was hoped in the present investigation to determine the levels of the urinary estrogens during pregnancy in the domestic buffalo *"Bos (Bubalus) bubalis*, L.".

MATERIALS AND METHODS

Experimental animals: Six healthy adult buffaloes aged from three to five years were available at the Animal Research farm, Faculty of Agriculture, University of Cairo, Giza, Egypt. They were mated during October and December 1953. Urine samples were collected throughout the period of gestation (316 days) at weekly intervals from one animal and monthly collections were taken from the other animals. Two virgin buffalo-heifers of three years old were available to serve as controls, the first was immature while the second was showing normal estrus cycles every 21 days; this was ovariectomized.

The experimental animals were fastened and fed on Egyptian clover during the period between December and May; throughout the rest of the year they were fed on cotton-seed cakes, rice bran, and wheat bran. This experiment lasted from January till November 1954. Since the animals under investigation were lactating, the urine was collected only during six hours (from 6 A.M. to 12 A.M) on the day of collection. There is no marked change in the hormone production rate over the

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twenty-four hour period (Turner, 1929; Dow and Allen, 1949), therefore the time of the urine collection would have no appreciable effect on its concentration. The urine collection equipment consisted of a heavy curved rubber tube held in position by waxed strings passed and tightened around the back and abdomen of the animal. The method of attaching the urine conduit has caused little inconvenience or discomfort to the buffaloes. When urine specimens were small in volume, another sample was secured on the following day. Measurement of urine volume was recorded, and a thin layer of toluene was added to the urine during collection periods to serve as a preservative.

Determination of estrogens. — The method used for determination of estrogens is based on the Kober test as modified by Cohen and Marrian (1947). An acid hydrolysis of the urine to obtain the hormone in the free form was followed by several toluene extractions, the combined extracts were washed with sodium carbonate 10 %. The carbonate washed toluene phase was extracted with 2N sodium hydroxide, the alkaline solutions were then covered with ethyl ether and acidified with hydrochloric acid to a pH of 3.0. The free estrogens were recovered by evaporating the organic solvent to dryness. 0.4 cc. of alcoholic solution of sample containing 10-50 μg of estrogens were allowed to react with concentrated sulphuric acid in the presence of heat. After cooling, the addition of definite amount of diluted sulphuric acid (25+75), boiling, and cooling again the characteristic pink color is developed. Readings taken on "Evelyn" colorimeter at 515 and 420 mμ permit calculation of estrone values corrected for the brown coloration caused by impurities. Estradiol and estrone were calculated as estrone.

Recovery test. — Large amounts of pure crystalline estrone solved in ethanol were added to free-estrogen urine (from ovariectomized buffalo) and determined colorimetrically to test the dependability of the method followed in the present investigation.

Colorimetric versus fluorimetric assay figures. — The alcoholic extracts of urine specimens collected from four experimental animals at different stages of pregnancy were processed colorimetrically and fluorimetrically, the fluorescence was read in a Coleman electric photofluorometer model 12B, the B-3 and PC-9A filter combination was used (Bates and Cohen, 1950).

RESULTS

When urinary estrogens of the pregnant buffaloes were fractionated, estradiol and estrone were found while estriol was absent. The first detectable amount of estrogen was during the third month of gestation at an average level of 37.5 μg per liter, this was followed by an increase until the 8th month. During the 9th month the reddish brown color developed in the blank was higher than that of urine samples from pregnant buffaloes, therefore the estrogen titers could not be measured. During the tenth month, the hormone level was maintained at an average of 193 μg per liter until the last fifteen days prior to parturition when there was no detectable estrogen (Table I). The maximum amount of estrogens excreted did not always occur at the same time in the different

TABLE I

Excretion of urinary estrogens during pregnancy

Stage of Pregnancy (Months)	Microgram Per Hour		Milligram Per Day	
	Range	Mean	Range	Mean
1st.	Nil	Nil	Nil	Nil
2nd.	Nil	Nil	Nil	Nil
3rd.	25-50	37.5	0.7-2.0	1.350
4th.	50-216	123.8	0.7-5.7	3.662
5th.	165-300	105.2	0.2-6.5	1.688
6th.	50-400	186.6	1.4-7.1	3.453
7th.	110-375	201.2	0.6-4.7	2.798
8th.	100-625	340.6	1.4-7.5	4.155
9th.	—	—	—	—
10th.	75-300	193.7	0.9-3.4	2.160

buffaloes (fig. 1), in the animals Nos. 1, 2, 3, the peak of estrogen excretion occurred during the 6th, 7th, and 8th month of pregnancy successively. Great variations existed in the amounts of estrogens excreted by the different buffaloes at the same stage of pregnancy. The estro-

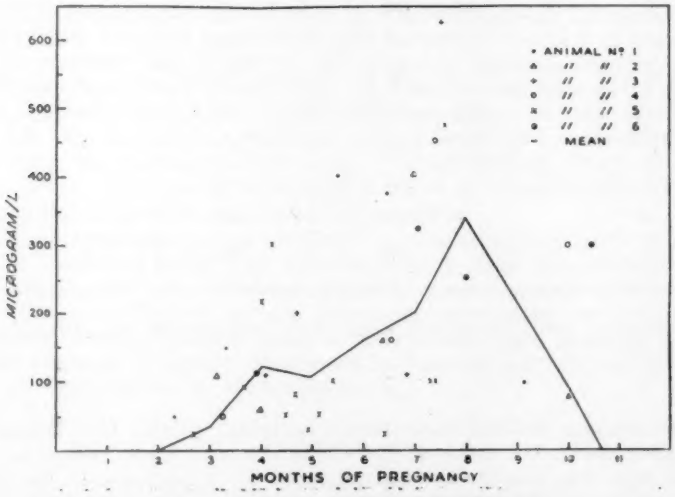

FIG. 1.— *Estrogen excretion per liter of urine during buffalo pregnancy.*

gen concentration for the buffalo Nº 2 and the buffalo Nº 3 in September varied from 75 to 300 μg per liter of urine although specimens of urine were collected within the same week of pregnancuy and the calendar month.

The recovery of estrone added to free-estrogen urine ranged from 95.50 % to 85.80 % with an average of 90.73 %. The metric values of

TABLE II

Estrogen level as determined by colorimetric and fluorimetric assay

Animal Nº	Days Pregnant	Estrogen Level ug./L.	
		Colorimetric	Fluorimetric
1	101	150	30
2	124	60	5
3	141	200	35
4	130	Nil	Nil
5	178	Nil	22
6	149	16.5	14
5	186	Nil	13.5
5	193	50	20
5	200	Nil	10
1	131	Nil	5
2	153	Nil	10
3	169	Nil	20
4	159	Nil	30
6	177	Nil	45
5	215	100	10
55	222	100	31.5
5	229	475	50

Colorimetric and Fluorimetric assay have been carried out using the same urine samples.

the same samples showed inconsistent variation (Table II). Towards the end of pregnancy, the color of the urine changed from yellow to rather greenish one, this was always accompanied by an increase in the interfering chromogens which obscured the pink color produced by estrogens and sulphuric acid. The greening in the urine also appeared in the ova-

riectomized animal in August and caused a considerable increase in the reddish brown color.

Discussion

The urinary estrogens recovered from pregnant animals occur in different forms, proportions and physiological activity according to the species. In the buffalo, estradiol and estrone are detected while estriol is absent. The same holds true for the cow (Hansel, 1954) and ewe (Beck, 1950). In the pregnant mare, however the urinary estrogens are estrone, estrone sulfate, alpha and beta-estradiol, equilin, equilenin, alpha-dihydroequilenin and hippurin (Girard et al., 1932a, b; Wintersteiner et al., 1936). It is possible that there are evolutionary trends in the hormonal system during pregnancy and this may be associated with the phycio-anatomical classification of the placentation. It may be concluded that the titers for estrogens in the buffalo urine are too low to have any quantitative significance except to indicate that the buffalo does not excrete significant amounts of estrogens (estradiol and estrone) via the urinary tract. The low level of urinary estrogens in the buffalo may be due to rapid metabolism in this species. It is possible that estrogens in the buffalo are mainly excreted in the feces as occurs in the cow (Levin, 1945; Pearlman et al., 1947, *19*, 48).

Further modification in the assay method adopted is needed to increase the validity of the present physiochemical technic for estrogen determination in urine samples during late pregnancy. The change of the urine color from yellow to greenish one at this period is always accompanied by a considerable increase in the brown pigments which have the general configuration of the steroid molecule but is devoid of its physiological activity. It is well known that estradiol and estrone give slightly different intensity of color or fluorescence, as the present determinations are made on mixture of both hormones, hence it is not supposed that the accuracy data is 100 %. Fluorimetric determination of estrogens would have been more useful than colorimetric one because of the small amounts of estrogens found in the buffaloes urine. It must be mentioned here that the fluorimetric methods require fairly pure concentrates from urine while the buffaloes urine contains much phenols which increase the fluorescence; this would invalidate the use of fluorimetric technic for estrogen determination in the buffaloes urine.

The inconsistent values obtained by measuring estrogens of the same samples colorimetrically and fluorimetrically may be attributed to the interfering substances which are found in great amounts in the buffaloes urine. Determination of urinary estrogens excreted by the buffalo during pregnancy using a chemical assay is insignificant as a pregnancy diagnostic test since it is usually diagnosed in this animal after some 90 days from the last service by rectal palpation.

Summary

Urine samples were collected from pregnant buffaloes at monthly intervals and processed for determination of estrogens gy using a physicoche-

mical method based on the Kober test and modified by Cohen and Bates (1947, 1949).

1. Estradiol and estrone were detected and determined as estrone whilst estriol was absent.

2. Estrone was first detected during the third month of gestation at an average value of 37.5 μg per liter of urine. Maximum estrogen excretion was 340.6 μg per liter of urine at the eighth month, this was followed by gradual fall at term. No estrogens were detected in the last 15 days prior to parturition.

3. There was marked individual variations in the concentration of urinary estrogens at identical stages of pregnancy. Period of maximum excretion was not uniform in all individuals.

4a. Recovery test of crystalline estrone from the free-estrogen buffalo urine averaged 90.73 %.

4b. Hormone titers determined colorimetrically varied inconsistently with fluorimetric values.

5. The urine color during late pregnancy changed from yellow to greenish one. This was accompanied by an increase in the interfering chromogens.

ACKNOWLEDGEMENTS

Thanks are due to Professor A. L. Badreldin, Faculty of Agriculture and Dr. H. Maghrabi, Ministry of Agriculture for very useful suggestions and criticism.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

Córdoba, August 4th, 1955

Toxicity of N-methyl-isocoridine chloride. BY E. MOISSET DE ESPANES. *Instituto de Investigación Médica "Mercedes y Martín Ferreyra" - Casilla de Correo 389 — Córdoba - Argentina.*

N-Methyl isocoridine, a quaternary base of the apomorphine group, presents toxicological characteristics resembling those of the other ternary alkaloids of this group. It produces respiratory failure before heart failure and it induces hyperexcitability in the rabbit and rat and hypoexcitability in the toad.

The LD 50 for the rat, by intraperitoneal injection, is 10.9 ± 0.9 mg/kg and the formula of the regression line is $y = 8.3x - 8.6$. Mesantoine and coramine increase the tolerance for N-methyl isocoridine, while lobeline and the barbiturates do not modify it.

Distribution of alkaline phosphatase in different proteic fractions separated by electrophoresis. BY S. TALEISNIK, S. PAGLINI AND VIOLETA ZEITUME. *Instituto de Investigación Médica "Mercedes y Martín Ferreyra" - Casilla de Correo 389 - Córdoba - Argentina.*

The distribution of alkaline phosphatase in the different proteic fractions separated by electrophoresis was studied in the serum of rats. In the normal fasting animals the enzyme was found in the beta and $alfa_2$ fractions in equal proportions. In the hepatic regeneration following partial hepatectomy and in animals fed with fat, the beta globuline-phosphatase increased. While in animals with ligature of the common bile duct it was the $alfa_2$ globuline phosphatase which increased; furthermore there appeared some enzymatic activity in the alfa, globuline fraction.

Determination of liver mass by bromosulfalein in normal rats. BY S. TALEISNIK. *Instituto de Investigación Médica "Mercedes y Martín Ferreyra" - Casilla de Correo 389 - Córdoba - Argentina.*

Liver mass was determined by bromosulfalein in normal rats. Its value can be calculated by the formula $Lm = 1.27x + 0.11$ knowing the body weight.

With the values found in rats, rabbits, dogs and men it was found to correspond to the exponential formula $Lm = 0.02 + x^{0.67}$.

Effect of N-methyl-isocoridine on the pupil. BY E. MOISSET DE ESPANES. *Instituto de Investigación Médica "Mercedes y Martín Ferreyra" - Casilla de Correo 389 - Córdoba - Argentina.*

N-methyl isocoridine, an alkaloid from the bark of Fagara coco (Gill. Engl.) administered by way of the lymphatics, produces intense and persistent miosis in the toad (*Bufo arenarum* Hensel). Its effect is not modified by ether anesthesia or the destruction of the central nervous system. It does not prevent pupillary reactions to the different intensities of light or to adrenaline. Acetlycholine does not prevent the effects of N-methyl isocoridine; adrenaline delays them. The topical application on the eye is ineffective.

The size of the rabbit's pupil is not affected, either by intravenous or local administration.

Buenos Aires, October 6th, 1955

Positive Gomori substance and antidiuretic activity of the choroid plexus of the third ventricle of the toad. BY J. M. TRAMEZZANI AND JULIA URANGA. *Instituto de Biología y Medicina Experimental, Costa Rica 4185 - Buenos Aires - Argentina.*

The choroid plexus of the third ventricle of the toad brain had positive Gomori substance in the epithelial cells and in the stroma of villosities. The biological titration of the antidiuretic action of this plexus showed an activity equivalent to 1014 milliunits of Pitressin per mg of plexus.

No antidiuretic activity was observed in the choroid plexus of the fourth ventricle, in which very few positive Gomori granulations were seen in the epithelial cells of the choroid villosities.

Effect of thyroidectomy by I^{131} on the alloxan diabetes in the dog. BY B. A. HOUSSAY, A. B. HOUSSAY AND A. F. CARDEZA. *Instituto de Biología y Medicina Experimental, Costa Rica 4185 - Buenos Aires - Argentina.*

Destruction of thyroid by I^{131} has disminished slightly the hyperglycemia and requirement of insulin of three dogs with alloxan diabetes. This action was transitory and diabetes was not cured nor markedly alleviated. The animals had a typic myxedema.

Uptake of radioactive S^{35} by the mucopolysaccharides of adult connective tissue, embryonary and cultures "in vitro". BY R. E. MANCINI, C. NÚÑEZ AND E. S. DE LUSTIG. *Comisión Nacional de la Energía Atómica. Dto. de Medicina y Biología. Buenos Aires - Argentina.*

A radioautographic study of the uptake of S^{35} (sodium sulfate) in different dosis and periods of time, has been carried out in tissue culture of chick fibroblasts and in mesenchyme, cartilage and connective tissue of chick embryo and adult rats. The tissues were fixed in Bouin's fluid and formol-alcohol mixture.

Radioautographic technique using the stripping-film method was applied to the histological sections.

To investigate the nature of the substance responsible for the uptake of S^{35}, another series of histological sections were incubated with barium hydroxide, hydrocloric acid, testicular hyaluronidase, colagenase and elastase, and then the slides were submitted to the radioautographic method.

It was observed that: 1) in tissue culture "in vitro", a moderate uptake of S^{35} in the cytoplasm of growing fibroblasts during the firsts hours and then until 48 hs in the intercellular substance. 2) Slight incorporation in the hystiocytes at two hours after that time until 72 hs, in the intercellular substance of mesenchyme in chick embryo; there was an increase in a pericellular disposition in precartilagenous and preconnective tissue areas. In cartilage tissue already developed, the uptake of S^{35} was more intense and predominantly in the cells at one hour, and then increasing in the matrix. 3) In adult rats, intense and progressive accumulation from three to 24 hs in cartilage, mast cells, and less intensely in the interfibrillary substance of the aortic and pulmonary walls, adventitia of arterioles, chornea matrix, sclerotica, tendons, aponeurosis, cardiac valves, upper chorion of skin, loose and dense connective tissue of the submucosa and stroma of various organs. After that time until 96 hs, no change was observed in the accumulation of the radioisotope. The uptake by the cells in the first hours and later in the intercellular substance was clearly seen in hyaline cartilage, but it was not so evident in the connective tissue cells. 4) Incubation of the slides with barium, hydrochloric acid and hyaluronidase removed the S^{35} of the tissue culture of fibroblasts, mesenchyme and cartilage of chick embryo. A similar effect was obtained in the cartilage, mast cells and connective tissue of the rats, with barium hydroxide, but not with hyaluronidase or hydrochloric acid.

Collagenase or elastase had no effect on S^{35} of the mesenchyme, cartilage or connective tissue. 5) Only barium hydroxide removed the radioisotope incorporated in the mucous glands and mucinous epithelium. The S^{35} accumulated in lesser amount in the kidney tubules, liver cells, or other organs was not affected by the incubation with the different substances used.

Buenos Aires, November 3rd, 1955

Study of diuresis in hemidecorticate rats. BY M. R. COVIÁN AND JULIA URANGA. *Instituto de Biología y Medicina Experimental, Costa Rica 4185 - Buenos Aires - Argentina.*

Rats which had been sumitted to the extirpation of one cerebral hemisphere had certain alterations in the excretion of water.

1) They had a delay in the urinary excretion with one load of water, and this difference was more pronunced with two loads of water.

2) This delay also appeared when the rat was loaded with 0.2 % NaCl solution, but disappeared with two loads of NaCl.

3) The antidiuretical activity of the urine was similar in normal and operated rats, both when water was either given *ad libitum* or withheld during the previous 48 hours.

4) There was no difference between operated and normal rats in the intake of water and the excretion of urine when given free access to water following normal hydration or a period of 48 hours of water restriction.

Transplantation of experimental ovary tumors. BY E. FELS AND N. DI FONZO. *Instituto de Maternidad. Dirección Nacional de Asistencia Social. Buenos Aires - Argentina.*

Pieces of ovarian tumours produced by intrasplenic graft or ligation of the ovary, were transplanted to 23 castrated male and female rats, to the spleen, liver and kidney. Twelve of the transplants were successful. The luteomatous structure of the regrafts can be modified, resulting in a tumour of granulosa cells or a tubular adenoma, with preservation of the estrogenic function. The malignant degeneration of a transplanted thecoma was observed once, in another case, a luteoma, cartilaginous tissue appeared, with loss of the estrogenic function in both cases.

Spermiation "in vitro" by water and saline solutions in the toad. BY H. J. SUER BOERO, *Arenales 1935 - Buenos Aires - Argentina.*

The author described an "in vitro" method to study spermiation in the *Bufo arenarum* Hensel. He tried to find out the threshold of spermiation using different concentrations of sodium chloride and other solutions.

It was found that the beginning of spermiation depends on temperature, hypophysectomy and fasting.

Spermiation is produced by hydratation of Sertoli cells. It is provoked by distilled water and hypotonic solutions, different solvents and substances.

Cardiovascular effect of butylscopolammonium. BY V. H. CICARDO AND A. O. VON DER BECKE. *Centro de Investigaciones Fisiológicas. Hospital Tornú, Buenos Aires - Argentina.*

Butylscopolammonium paralyzes in dogs the peripheral portion of the vagus nerves and produces the inversion of the hypotensive effect of acetylcholine resembling atropine in this action. As methonium salts, butylscopolammonium has no gangliopectic action since it appears not to influence arterial hypertension arising through electric excitation of cerebral cortex or spinal cord.

As a rule, the pressure effect of adrenalin and noradrenalin is reinforced by butylscopolammonium. There are no changes in the hypertension produced by excitation of the sympathetic nerves of the liver.

Factors modifying the sensitivity of succinoxidase for BAL's oxidation. BY A. O. M. STOPPANI AND J. A. BRIGNONE. *Instituto de Química Biológica, Escuela de Medicina, Universidad de Buenos Aires - Argentina.*

1º) Succinoxidase inhibitors (phenylurethane, antimycine A) which dissociate succinoxidase activators (phosphate ions, versenate) which promote closer association of succinatedehydrogenase and the cytochromes, effect directly Slater's BAL-sensitive factor.

2º) Competitive inhibitors of succinate-dehydrogenase do not prevent succinoxidase inactivation through BAL oxidation.

PROCEEDINGS OF THE SOCIEDAD DE BIOLOGIA DE SANTIAGO (CHILE)

April 26th, 1955

Adaptative chromosomal polymorphism in certain south american species of Drosophila.* BY D. BRNCIC. *Instituto de Biología, Cátedra de Biología. Escuela de Medicina. Universidad de Chile.*

Dobzhansky, Burla and Da Cunha (Amer. Nat. *84:* 229, 1950) and Da Cunha, Brncic and Salzano (Heredity *7:* 193, 1953) have shown, in several south american species of Drosophila, that there exists a correlation between the amount of chromosomal polymorphism and the diversity of the habitat in which a population lives. The greater the ecological versatility of a population, the greater the chromosomal polymorphism, and vice-versa. The distribution of the gene arrangements in Chilean populations of Drosophila immigrans (Sturtevent) is very instructive for the understanding of this phenomenon. Chilean populations of Drosophila immigrans show variations of the gene arrangement only in the second chromosome. Besides the "Standard", three different varieties of gene sequences, due to inversions, have been found. One of them is identical to the one described by Freire Maia et al. (Dusenia *4:* 303, 1953 in Brazil). Although the mean number of heterozygous inversions is low, it seems to be possible to establish a relationship between the type of habitat and the chromosomal polymorphism. In the populations from northern Chile, which live under the stringent ecological conditions of the desert, besides "Standard", only one gene arrangement is found. On the other hand, in the South, where the species occupies the rich habitat of the rain forest, besides "Standard", there are at least three different gene arrangements. Although D. immigrans is to a certain extent, a domestic species, it forms local populations, races, which differ from each other by the gene arrangements in their chromosomes. These racial differences evidently arose in response to the different ecological conditions and are maintained by the geographic isolation between the populations.

(*) This work has been supported in part by grants awarded by the Rockefeller Foundation.

Action of sodium gentisate on Schultz-Dale phenomenon and anaphylactic shock. BY C. MUÑOZ, S. ROZZI AND E. SERRANO. *Laboratorio de Farmacología Experimental, Escuela de Medicina. Universidad de Chile.*

The effects of sodium gentisate on the Schultz-Dale phenomenon tested in isolated guinea pig uterus and on the anaphylactic and histaminic shocks in guinea pigs were studied. Horse serum was used as sensitizing antigen. Sodium gentisate in concentrations ranging from 1.0 to 3.0 mg/ml prevented the Schultz-Dale phenomenon in 53 to 80 per cent of the sensitized guinea pig uterus in which the control horn reacted positively to horse serum. The same concentrations of this substance were unable to inhibit uterine contraction induced by histamine (0.4 microgram/ml).

Sodium gentisate administered intraperitoneally in doses of 0.2, 0.4 and 0.8 grams, 20 minutes before the injection of the active dose of serum, protected from anaphylactic shock 23, 67 and 60 per cent of sensitized guinea pigs respectively. Fifteen

days later 5 of the surviving animals received a new injection of horse serum, without previous administration of gentisate, and all died with typical anaphylactic shock.

Administration of sodium gentisate intraperitoneally in doses of 0.4 and 0.6 grams did not protect against histamine shock 27 out of 28 guinea pigs.

These results are consistent with the idea that gentisate interferes in the antigen-antibody reactions, as it has been suggested for salicilate.

May 10th, 1955

Antitumoral action of iodine compounds. BY F. OBERHAUSER, H. CROXATTO, M. GAILLARD AND V. SILVA. *Laboratorios de Química y de Fisiología. Instituto Pedagógico. Universidad de Chile.*

Mesencephalic control of muscle proprioceptors. BY R. GRANIT AND B. HOLMGREN. *Instituto Nobel de Neurofisiología, Karolinska Institute, Estocolmo. Instituto de Fisiología. Universidad de Chile.*

June 28th, 1955

Blood glutathione and vitamin B_{12} in dystrophic infants. BY F. MONCKEBERG, M. PERRETTA AND P. BERHO. *Laboratorio de Pediatría. Escuela de Medicina. Universidad de Chile.*

"In vitro" actions of DL-Glyceraldehyde and L-sorbose-1-phosphate on the hepatic and diaphragmatic glycogen of the rat. BY E. FIGUEROA AND H. NIEMEYER. *Instituto de Química Fisiológica y Patológica. Escuela de Medicina. Universidad de Chile.*

A system of linked antigenic factors in the mouse. BY G. HOECKER. *Instituto de Biología. Cátedra de Biología. Escuela de Medicina. Universidad de Chile.*

The H-2 locus in the mouse is responsible for blood group II and at the same time for histocompatibility of certain tumors in transplantation experiments (Gorer, Lyman and Snell, Proc. Roy. Soc. London, B, *135:* 499, 1948). A serological analysis has shown that it is also responsible for a complex system of isoantigens that are present both in red cells and tissues (Hoecker, Counce and Smith, Proc. Nat. Acad. Sci., *40:* 1040, 1954). Different inbred strains of mice have different combinations of antigenic factors. Some antigens are characteristic of certain strains and others are shared in common by several strains.

Three mutations at this locus have been shown each to change two antigenics factors at once. This fact speaks more in favour of different alleles being responsibles for the production of all the antigenic factors than the hypothesis of closely linked genes.

Experiments using co-isogenic resistant strains have shown that it is enough for a transplant to differ in one antigenic factor with its host to induce a homograft reaction.

July 12th, 1955

Effects of vitamin B_{12} on the metabolism of carbohydrates and lipids studied in dystrophic infants. BY F. MONCKEBERG, M. PERRETTA AND P. BERHO. *Laboratorio de Pediatría. Universidad de Chile.*

The action of total extracts of neurohypophysis on the sodium excretion in rats. BY R. ROSAS AND H. CROXATTO. *Laboratorio de Fisiología. Universidad Católica de Chile.*

The possibility of destroying the vasopressor activity of neurohypophysis extracts under trypsin would solve the problem whether their sodium excretion activity in due to vasopressin or to oxytocin (Croxatto, H., Rev. Med. Aliment., *5:* 300, 1942) (Lawler, H. C. and V. du Vigneaud, Proc. Soc. Exp. Biol. & Med. *84:* 114, 1953). With this point in mind, semipurified extracts of oxen neurohypophysis were incubated with trypsin alone or associated with carboxypeptidase. The vasopressor and oxytocic activity of these extracts were controlled and the excretion of urine and sodium compared to control groups that were injected with 0.9 % NaCl solution.

a) 30 fasting rats that had free access to water were injected subcutaneously (0.1 ml) and diuresis was controlled during the following 12 hours.

b) 45 fasting hyperhydrated rats (5 % of weight) were injected intraperitoneously and diuresis was controlled every 15 minutes. A special group (11 rats) was injected by way of the caudal vein.

Digestion with trypsin alone or associated to carboxypeptidase destroyed completely the hipertensive activity of the extracts, without a significant decrease of the oxytocic action.

In rats with free access to water during the following 12 hours after injection of the incubated extracts with and without enzyme, a significant increase of water and sodium excretion was observed. Sodium excretion was lower in those rats that received the extracts incubated with enzymes.

The extract had the typical antidiuretic activity on the hyperphydrated rat and it was considerably decreased with the enzymes. The differences was more evident after intravenous administration.

August 2nd, 1955

An enzymatic method for the titration of arginine. BY J. CABELLO AND V. PRAJOUX. *Instituto de Química Fisiológica y Patológica. Universidad de Chile.*

A method is proposed in order to titrate arginine in protein hydrolisates by an enzimatic procedure.

0.5 g of protein is refluxed with 5 ml of 6 N HCl during 24 hours, neutralized with 6 N NaOH to pH 6.0 and diluted to 100 ml. For arginine titration, 1 ml of this dilution is mixed with 1 ml of distilled water, 0.04 ml of 0.2 M $MnSO_4$ and 1 ml of enzymatic material and is incubated 30 minutes at 37° C. The enzymatic material may be a young rat liver homogenate 1:100 in glycine buffer pH 10.14 or a purified preparation in glycerol, also buffered to pH 10.14. After the incubation period, proteins are removed with 1 ml of 24 % metaphosphoric acid and centrifuged. Urea is citrate in the supernatant. Simultaneously, a blank is prepared with 2 ml of distilled water, Mn and enzyme, and the standard which contains 1 ml of L (+) arginine chlorhydrate (50 mg/100 ml), 1 ml of water, Mn and enzyme.

The formed urea is measured colorimetrically by the Archibald technique (J. biol. Chem., *157*: 507, 1945) employing 1 ml of supernatant, 4 ml of sulphuric-phosphoric reagent and 0.25 ml of α isonitrosopropiophenone solution.

Arginine HCl (mg per gram protein) is calculated as follows:

$$\frac{0.5\ R_t \quad V}{R_s \quad m}$$

where R_s is the standard reading (Klett-Summerson, 540 mμ); R_t is the test reading; V, the total hydrolisate volume in ml and m the digested protein mass in grams.

Arginine is stable towards acid hydrolisis. The standard error in titrations with solutions of the amino acid is ± 4 %. The results obtained with gelatine, peptone, casein and protamine sulphate, expressed as percentage of argininic N to total

protein N, are consistent with those reported in the literature. Arginine added to proteins is quantitatively recovered with the same error.

The advantages of the proposed method compared with Jansen (Jansen, B. C. P., Arch. Neerl. Physiol., *1*: 618, 1917) and Hunter and Dauphinee methods (Hunter, A. and Dauphinee, J. A., J. biol. Chem. *85*: 627, 1929-30), are the simplification of acid hydrolisis and the shortening of incubation time (from 24 hours to 30 minutes). Increased senstivity and precision are obtained by activating arginase with Mn and by employing Archibald procedure for determination of urea. The proposed method can be easily adapted to any particular requirement.

Effect of some self-selection conditions on the voluntary alcohol intake of rats. By J. Mardones, N. Segovia-Riquelme, A. Hederra and F. Alcaíno. *Instituto de Investigaciones sobre Alcoholismo y Laboratorio de Nutrición del Instituto de Educación Física. Universidad de Chile.*

Changes in the voluntary alcohol intake by rats depleted of factor N_1 under various self-selection conditions were studied.

The self-selection of solid sucrose and of the nonsucrose moiety of the basic diet did not significantly change the intake of alcohol. A dietary supplement of dried liver decreased the alcohol intake in most of the animals. This decrease was not correlated with changes in the intake of solid food.

Changes in the proportion of sucrose in the diet from 64 per cent to 81 per cent and to 29 per cent did not significantly alter the intake of alcohol.

The offering of a third fluid choice in the from of sucrose or dextrose solutions induced a significant decrease of alcohol intake in all the rats, confirming Lester and Greemberg's results (Quart. J. Stud. Alc. *13:* 553, 1952). Concentrations of the sugar solutions ranging from 10 per cent to 70 per cent did not differ in their influence on the alcohol intake.

The offering of a third choice of a solution of pure B vitamins, for which rats exhibit a clear preference, did not significantly change the alcohol intake.

Enzymatic synthesis of phosphocreatine in the absence of adenylic nucleotides. By O. Cori. *Instituto de Fisiología. Universidad de Chile.*

These observations deal with an enzymatic system of the rat muscle which synthesizes phosphocreatine starting from creatine and inorganic phosphate and utilizing fructose-1.6-diphosphate as substratum. This system requires in addition magnesium and diphosphopyridine nucleotide. This same system does not sterify inorganic phosphate in the presence of glucose and hexokinase, unless adenosine diphosphate is added in catalytic quantities. In the absence of adenylic mononucleotides, glucose-hexokinase or myosine does not inhibit the synthesis of phosphocreatine, while they are inhibitors in the presence of such nucleotides. It is possible that diphosphoglyceryl-phosphate could phosphorylate creatine directly and not through the adeninmononucleotides.

Relation between blood coagulation and vasoconstriction in normal and in hemophilic subjects. By R. Honorato and A. Arenas. *Laboratorio de Química. Escuela Dental. Universidad de Chile.*

Our results indicate that the vasoconstrictor action of pitressin instilled directly on the frog's kidney (Bufo chilensis) depends on what has been previously injected into the frog. Glomerular circulation stops 15 to 30 sec after pitressin instillation in animals injected with normal plasma, saline or in non-injected animals. In animals injected with hemophilic plasma this reaction has a delay of 10 minutes at least, and glomerular circulation may not stop at all in more than 50 % of the cases.

Storage of plasma at -12° C normalises the hemophilic test in 9 or 10 days. This indicates that disturbances of vasoconstriction ore not caused by a deficiency of the anti-hemophilic factor.

On the other hand, normalization of this test can also be obtained by adding calcium chloride to the hemophilic plasma, previously to the injection, without

normalization of blood coagulation. Moreover, the injection or sodium oxalate, withouth plasma, transforms the normal reaction to pitressin into one of the hemophilic type. These facts indicate that an ionic unbalanced hemophilic plasma may be producing the abnormal reaction of the glomeruli and of the arterioles of the frog. It is improbable that an inhibitor in the hemophilic plasma could explain these results.

August 16th, 1955

Sympathetic spectrum of skin and muscle innervation. BY W. R. LOEWENSTEIN. *Department of Zoology. University of California, Los Angeles - U. S. A.*

Mechanism of sympathetic neuromuscular facilitation. BY O. F. HUTTER AND W. R. LOEWENSTEIN. *Wilmer Institute. Johns Hopkins University, Medical School, Baltimore - U. S. A.*

Sympathetic influence on the small-nerve motor system. BY O. F. HUTTER AND W. R. LOEWENSTEIN. *Wilmer Institute. Johns Hopkins University, Medical School, Baltimore - U S. A.*

Method for detecting hemophilic carriers. BY R. HONORATO AND A. ARENAS. *Laboratorio de Química. Escuela Dental. Universidad de Chile.*

In frogs previously injected with hemophilic plasma, the glomerular circulation either is unimpaired or it is arrested after a long period of time if pitressin is directly instilled on the kidney. This characteristic reaction to pitressin makes these animals clearly different from those injected with normal plasma.

In this work we studied the possibility of normalizing the pitressin test of hemophilic subjects by mixing normal and hemophilic plasma. Also the behaviour of carrier plasma was studied by mixing it with hemophilic plasma.

The capacity of normal plasma to normalize hemophilic plasma (1 part normal and 39 parts hemophilic plasma) was constant. Carrier plasma was incapable of normalizing it. This enabled us to detect carrier plasma.

Furthermore, dilution of carrier plasma established striking differences between normal and hemophilic subjects. The normal plasma behaved similary whether injected pure or diluted (25 %). The hemophilic plasma behaved abnormaly both concentrated and diluted. Exceptionally it showed some improvement with diluted plasma. The undiluted carrier plasma reacted as normal plasma on 60 % on the cases and as hemophilic plasma when diluted. In 40 % of the cases it reacted as hemophilic both with concentrated and diluted plasmae.

August 23th, 1955

Creatine phosphorylation. Properties of the enzymatic system. BY O. CORI AND F. ABARCA. *Instituto de Fisiología y Departamento de Zoología del Instituto Pedagógico. Universidad de Chile.*

These observations deal with the effect of fluoride and of monoiodoacetic acid on the synthesis of phosphocreatine in the absence of adenylic nucleotides. Both substances are strong inhibitors. Synthesis of phosphocreatine starting from 3-phosphoglyceric acid requires the presence of catalytic quantities of ATP. This is not the case if one start from fructose-diphosphate. The enzyme is capable of producing the arsenolysis of phosphocreatine only in the presence of reduced diphosphopyridine nucleotide and of phosphoglyceric acid. This emphasizes the concept that phosphoglyceryl-phosphate is the precursor of phosphocreatine.

Influence of potassium iodide on the atherosclerosis induced in chickens by diethylstilbestrol alone or associated with cholesterol-rich diet. BY J. CEMBRANO, D. RIQUELME, V. ACOSTA AND J. MARDONES. *Laboratorio de Farmacología. Escuela de Medicina. Universidad de Chile.*

The incidence of macroscopic atherosclerosis in the aorta and its branches induced in chickens of both sexes by diethylstilbestrol pellets (25 mg monthly) alone or combined with cholesterol-rich diet, was studied. The influence of KI (75 mg per kilogram body weight daily given in the drinking water) on the atherosclerosis of chickens submitted to the mentioned conditions, was also studied. The birds were killed for autopsy between 80 and 140 days.

The incidence of atheroma in birds treated only with stilbestrol was similar in both sexes (males: 5/20; females: 5/22). No macroscopic lesions were observed in males and females with gonads greater than 0.3 % of the body weight.

The incidence of atherosclerosis in chickens treated with stilbestrol and fed with cholesterol-rich diet was significantly higher than in those treated only with stilbestrol in both sexes (males: 16/21; females: 17/28) showing that the protection against cholesterol-rich diet observed in pullets (Cembrano, J., Del Solar, V., Muñoz, C. Ratinoff, E. and Mardones, J. Ciencia e Investigación, *8:* 559, 1952) was inhibited by stilbestrol.

The administration of KI did not influence the incidence of stilbestrol atherosclerosis on birds fed on basic diet (males: 7/21; females: 3/18), and in cockerels treated with stilbestrol and cholesterol-rich diet (17/19). On the contrary, KI induced a significant protection among the pullets receiving stilbestrol and cholesterol-rich diet (5/22, $P = 0.02$).

Influence of dietary lipids and of potassium iodide on the changes of liver lipids induced by diethylstilbestrol in chickens. BY A. PENNA, J. CEMBRANO AND J. MARDONES. *Laboratorio de Farmacología. Escuela de Medicina. Universidad de Chile.*

Liver lipids, their iodine number and liver cholesterol were estimated in chickens submited to atherogenetic conditions (diethylstilbestrol pellets and or cholesterol-rich diet) associated or not with KI administration (75 mg/Kg body weigth daily). Basic diet was a commercial mashfood (lipids 4.1 per cent; iodine number 103; cholesterol 0.15 per cent). Cholesterol rich diet (lipids 14.0 per cent; iodine number 63.7; cholesterol 2.88 per cent) was obtained by mixing eigth parts of basic diet with one parte of dried cattle spinal cord.

The liver of the chickens fed either with basic or with fat-rich diet killed after 120 to 148 days of stilbestrol treatmente exhibited lipid content significantly higher than the normal. KI did not influence this effect in these chickens. Liver cholesterol was also significantly higher than the normal, but in the birds receiving basic diet it was than in those fed with cholesterol-rich diet. KI did not modify the liver cholesterol of cockerels receiving basic or cholesterol-rich diet. On the contrary, in the pullets KI induced a decrease of liver cholesterol. This decrease was not significant in femals receiving basic diet but significant ($P = 0.02$) in femals fed with cholesterol-rich diet.

The iodine number of liver fat was not affected by the different experimental conditions.

The birds killled after 120 days were classified according to the presence or abscense of macroscopic atheroma and no significant differences were observed between both groups of birds receiving cholesterol-rich diet. But among the birds receiving basic diet, liver lipids were significantly lower in those without lesions than in those with lesions. No differences were observed in liver cholesterol between birds with and without lesions.

The iodine number was not different in both groups of cockerels but it was significantly lower in pullets without lesions.

Birds receiving diethylstilbestrol treatment for 80 to 113 days did not differ from untreated chickens, concerning liver cholesterol, liver lipids and their iodine number.

August 30th, 1955

A simple device to measure continuous diuresis in rats. BY H. CROXATTO, J. REYES AND R. VERA. *Laboratorio de Fisiología. Instituto de Educación Física. Universidad de Chile.*

A simple device for observation and continuous measurement of urine excreted by rats anesthetized with alcohol and under hyperhydration has been developed.

The so called "continuous flow" device consists of two transparent polyethilene tubes having a length of 1.50 m and 1.14 mm diameter connected to a small cannula placed inside the urinary bladder. The tubes are set lengthwise upon a wooden bar calibrated in centimeters. The transparency of the tube allows seing the advance of the urine and a continuous measurement of urinary excretion. The linear centimeters advanced by the urine can be converted into cubic milimeters if the section of the tube is known. This device has proved very useful to measure the antidiuretic activity of extracts of the neurohypophysis. Variations produced by 0.01 mU of vasopressin are detected.

Sympathetic influence on peripheral mechanoreceptors. BY W. R. LOEWENSTEIN. *Department of Zoology. University of California. Los Angeles. U. S. A.*

Interactions of sympathetic effects on the facilitation and excitation of tactil receptors in a depolarized electric field. BY W. R. LOEWENSTEIN. *Department of Zoology. University of California. Los Angeles. U. S. A.*

September 13th, 1955

A comparative study on the excretion of water, Na, Cl and K in rats injected with vasopressin and oxytocin. BY H. CROXATTO, R. ROSAS AND L. BARNAFI. *Laboratorio de Fisiología. Universidad Católica de Chile.*

Previous investigations (Croxatto, H., Rosas, R. and Barnafi, L., 3rd Panamerican Congress of Endocrinology, in Press) have shown that the antidiuretic and diuretic activities of neurohypophysiary extracts are due to oxytocin. These findings agree with Frasser's work (Frasser, A. M., J. Physiol., 101: 236, 1942).

Due to the contradictory data available on this subject, a comparative study was made using highly purified extracts of oxytocin and vasopressin. The subcutaneous injection of a product containing 240 oxytocic U and 3 pressor U, at doses between 25 to 100 mU produces a great increase in water, Na and Cl excretion during 120 minutes following its administration. The rats had free access to water. This activity is mantained even after the incubation with trypsin either alone or associated to carboxypeptidase. Chymotrypsin induces the disapearance of this phenomenon and of the oxytocic activity as well.

The injection under the same conditions of a purified product containing 60 pressor U and 3, oxytocic U per ml, has a great antidiuretic and antinatriuretic activity. This activity does not decrease in proportion to the vasopressor activity of the product upon incubation with trypsin and chymotrypsin. Treatment of the extract with trypsin associated to carboxypeptidase is successful in destroying the hypertensive activity as well as the antinatriuretic and antidiuretic activities of the hormone.

September 27th, 1955

The effect of purified oxytocin and vasopressin upon sodium and water excretion in hypophysectomized rats. BY H. CROXATTO AND B. Z. MORANO. *Laboratorio de Fisiología. Instituto Pedagógico. Universidad de Chile.*

Purified oxytocin, almost free from vasopressin, administered at doses of 50

mU has no activity at all upon hypophysectomized rats that have previosly been submitted to diets with high sodium content as well as sodium deficient diets.

Vasopressin with a very small amount of oxytocin produces a decrease in water and sodium excretion in normal and hypophysectomized rats during four hours following the injection.

The administration of DCA (1-2 mg/day) and of DCA plus thyroxine (0.05 mg/day) to hypophysectomized animals does not reestablish the diuretic activity of oxytocin. It favours instead the excretion of sodium when the animals are fed on a diet containing high amounts of NaCl.

The administration of NaCl in the drinking water (2.5 ‰) plus the daily injection of DCA permit the oxytocic effect upon the excretion of water and sodium in the normal as well as in the hypophysectomized animals during four hours following the injection.

In the animals thus treated the injection of 50 mU of oxytocin administered 10 minutes before the injection of 10 mU of vasopressin is able not only to counteract the antidiuretic and the sodium excretion inhibitory activity of the latter but to produce the opposite effect.

Influence of several substrats on the anaerobic production of lactic acid by rat liver slices. BY H. NIEMEYER, G. GONZÁLEZ AND E. FIGUEROA. *Instituto de Química Fisiológica y Patológica. Universidad de Chile.*

October 4th, 1955

The role of decalcifying agents on the stability of platelets. BY R. HONORATO AND G. SCHINDLER. *Laboratorio de Química. Escuela Dental. Universidad de Chile.*

Am-coagulants that act by decalcification of the blood protect human platelets from destruction by glass contact if used at higher concentrations than those necessary to prevent blood coagulation.

0.2 M sodium oxalate and 0.2 M sodium nitrate (1 ml to 9 ml) added to the blood avoid platelet destruction. 0.1 M sodium oxalate (1 ml to 9 ml) prevents blood coagulation but does not prevent platelet destruction if blood is left in contact with glass for 45 sec. 0.025 M sodium oxalate (1 ml to 9 ml) impedes coagulation but does not protect platelets even against a glass contact of 15 sec. These results differ with those obtained when native plasma is used. Platelets do not seem to be affected by sodium oxalate.

The experiments reported in this work show that anticoagulants (decalcifying agents) do not protect the platelets against glass destruction by their decalcifying properties. Red cells increase the sensitivity of platelets to glass contact. These experiments show that platelets from oxalated or citrated blood are not equal to native blood platelets, agreeing with previous work (Schindler, S. G., Honorato, C. R., & Ivanovic, F. R., Bol. Soc. Biol. Stgo. (Chile), 10: 5, 1953).

Influence of red cells and of calcium on the resistance of platelets and on their affinity to a cation-exchange resin. BY R. HONORATO, R. VÁSQUEZ, G. SCHINDLER AND A. VELASCO. *Laboratorio de Química. Escuela Dental. Universidad de Chile.*

The resistance of platelets to destruction after contact with glass and their adherence to a cation-exchange resin (Amberlite IR-100-H) has been found different in whole-blood platelets as compared with those found in plasma without red cells.

Amberlite IR-100-H produced a retention of platelets in most cases when whole-blood was studied. It held only a few platelets when plasma was employed.

An important loss of platelets from native blood was also observed when a sample was maintained longer than usual in contact with a capillary pipette.

These results enable us to state that important differences occur between platelets from native blood and those from plasma without red cells if their rate of destruction

is compared. Near 60 % of platelets from native blood were destroyed and only 12 % from plasma without red cells

Neither calcium nor red cells by themselves can produce this phenomenon. Native plasma with normal calcium concentration and citrated blood with red cells (with no available calcium) neither destroyed nor retained their platelets. Apparently both the red cells and calcium ions must be present to produce retention.

November 8th, 1955

Accommodation of normal and pathological dental pulp nerve. BY F. VARGAS. *Instituto de Fisiología. Universidad de Concepción* (*).

Nerve accommodation was studied in 20 normal and 15 pathological teeth stimulated by seven exponentially increasing currents with time-constants of: 4.85, 9.75, 12.23, 18.75, 21.25, 37.50 and 42.50 msec.

In normal cases the slope of accommodation was 0.164 Rb/msec (S.E.: 0.008). This value is higher than Solandt's (J. Physiol. 85: 5P, 1935), and Kuggelberg's (Acta Physiol. Scand. 8: 24, 1944) data for human tissues. In pathological cases the slope of accommodation decrease to 0 048 Rb/msec (S.E.: 0.006). The difference between both groups is statistically significant: $P < 0.001$. Ohmic resistance of 24 teeth was measured at short intervals using a Wheastone bridge. Tooth resistance fell to 53.46 % of the initial value and became stabilized at this level.

Since accommodation is a linear function in normal and pathological pulp nerves it is feasible to derive it by comparing the thresholds for two different time-constants. This represents a simplified method to test excitability.

The great difference of accommodation between normal and pathological teeth allows the possibility of introducing this method in dental electrodiagnosis.

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The acculation of oxytocic substances in the tuber cinereum of hypophysectomized rats. BY V. SILVA, B. ZAMORANO, H. CROXATTO AND M. BECERRA. *Laboratorio de Fisiología. Instituto Pedagógico. Universidad de Chile.*

The increased concentration of oxytocic substances in the tuber cinereum of hypophysectomized rats has been demonstrated after sacrifying the animal from 5 to 14 days after hypophysectomy (Silva, V., Croxatto, H., Ampuero, O. and Aliste, N., 3rd Panamerican Congress of Endocrinology, 1954, in Press).

The oxytocic activity of the tuber of 59 control animals was studied. The oxytocic activity was also studied by using 67 animals previouly sacrified and later hypophysectomized, and 67 hypophysectomized animals which were sacrified half an hour after hypophysectomy.

The extracts were made by using equivalent amounts of tuber and ground with quartz sand. The pH was settled after their centrifugation. The oxycitocic activity was determined using isolated rat uterus.

Excepting 4 animals, oxytocic activity was higher when tuber of rats sacrified after hypophysectomy was used. No difference was found between the control group and the group which was hypophysectomized half an hour after being sacrified.

Antimetabolic effect of ethylene gylcol mono and dimethyl ethers on isolated rabbit ileum. BY J. ALDUNATE, R. MORALES AND J. MARDONES. *Laboratorio de Farmacología Experimental. Escuela de Medicina. Universidad de Chile.*

An antitumoral effect of ethylene glycol mono and dimethyl ethers on some experimental tumors has been observed by Oleson (Personal comunication). The chemical structure of these substances led to assume that they may act as antimetabolites on carbohydrate utilization.

The recuperation of isolated rabbit ileum made hypodynamic by working in Tyrode solution without metabolite, induced by glucose, lactate, pyruvate, acetate

and glycolate, was inhibited by both substances. For quantitative estimation of the antimetabolite effect, the recovery in the presence of metabolites with different concentrations of inhibitors was expresed as percent of the recuperation obtained with metabolites alone. The molar ratio antimetabolite/metabolite which induced a decrease of 40 to 60 per cent in the recuperation induced by glucose, pyruvate, lactate, acetate and glycolate were 15.2; 9.0; 2.7; 6.5; and 2.1 for the monomethyl-ether, and 4.7; 5.6; 0.9; 6.1 and 2.1 for the dimethyl ether, respectivelly.

The influence of doubling the metabolite concentration was studied for glucose and dimethyl ether and for glycolate and methyl ether. In both cases the antimetabolite concentrations inducing the same degrees of inhibition were also doubled.

Results are considered as an evidence that mono and dimethyl ethers of ethylene glycol acts as antimetabolites in different stages of carbohydrates metabolism. Lactic dehydrogenase seems to be specially affected. Dimethyl ether appears generally as a stronger inhibitor than monomethyl ether.

November 22th, 1955

Biological similarities. By E. Guerra and B. Günther. *Instituto de Matemáticas. Universidad de Concepción. Instituto de Fisiología. Universidad de Chile.*

Dehydrogenases in Trypanosoma cruzi. By M. Agosin. *Laboratorio de Parasitología. Universidad de Chile.*

Effects of dinitronaftol on the membrane potential of skeletal muscle fiber. By W. R. Loewenstein and A. Szent-Gyorgyi. *Wilmer Institute. Johns Hopkins Medical School. Baltimore. Institute for Muscle Research, Woods Hole - U. S. A.*

December 6th, 1955

Chromosomal variations in natural populations of Drosophila Mesophragmatica (*). By D. Brncic. *Instituto de Biología. Cátedra de Biología. Universidad de Chile.*

Drosophyla mesophragmatica, previously described for Peru and Bolivia (Duda, Arch. Naturgesch., *11:* 1, 1927) and for Brazil (Pavan and Da Cunha. Bol. Fac. Fil. Cien. Let. Univ. San Paulo, *86:* 20, 1947) is one of the most abundant species in Chile.

In the present paper, the author describes the metaphasic and polytenic chromosomes and gives a cytological map of the latter. The metaphasic plates show a pair of V chromosomes, three pairs of rods and one pair of dots. One pair of rod shaped chromosomes is heteromorphic in males, and corresponds to the sexual pair. In the salivary gland cells, one observes 5 long euchromatic arms, which converge towards the heterochromatic chromocenter, and one small element, which corresponds to the dot like chromosome. For the drawing of the cytological map a stock from "Arrayán" (Santiago) was taken arbitrarily as "Standard". The cytogenetic analysis of the progeny of females from various places of Chile and Brazil, as well as the study of hybrids between the "Standard" Arrayán stock and several other Chilean and Brazilian stocks, shows the following:

Brazilian populations differ from the Chilean ones, by being homozygous for two overlapping inversions in the sexual chromosome as respects "Standard".

Chilean populations appear polymorphic in relation to genic arrangements in the euchromatic elements, indicated in the map as II, III and V. In element II, besides Standard, there is an arrangement which results from two inversions, one included in the other. Besides "Standard", in each of elements III and V, one can observe a second genic sequence which results from three over-lapping inversions.

It is an interesting fact that neither in the autosomes nor in the X chromosomes were found the intermediate gene arrangements between "Standard" and the other chromosomes forms which are needed to understand the phylogeny of chromosomes "races" according to Dobzhansky and Sturtevant's hypothesis (Genetics, *23:* 28, 1938). As the frequency of inversions depends on the adaptive qualities of the genetic combinations which these inversions maintain free from recombination by crossing-over. The absence of these intermediate steps can be interpreted as being due to the fact that the selction process has maintained in the natural populations only these chromosome forms that were better adapted.

(*) This work has been supported in part by grants awarded by the Rockefeller Foundation.

Visual disturbances and recovery in monkeys with lesions of the temporal lobe. BY T. PINTO, G. SANTIBÁÑEZ AND L. SIMON. *Instituto de Fisiología. Universidad de Chile.*

Visual disturbances produced by lesions of the temporal cortex were studied in 6 monkeys by means of 3 color tests and 2 shape tests. Animals with lesions of the temporal neocortex were particularly deficient to those tests which require a higher degree of abstraction. Some monkeys showed deficiencies to all tests, whereas others failed only to the most difficult ones. Those animals with ventral lesions of the temporal lobe presented a paradoxical response. Tests which were simplest for the normal animals proved to be the most difficult ones after the operation. Recovery after the operation was studied in animals in which learning was studied 5 months after the operation. Visual discrimination and recognition of edible and non edible objects was performed. It was observed using visual discrimination tests that those animals which had no postoperatory experience their performance were as efficient as in those animals recently operated. On the other hand recognition of edible objects was better 5 months ofter the operation. It is concluded that recovery of visual habits is greatly influenced by experience.

Disturbances of olfactory discrimination after partial removal of the temporal lobe. BY G. SANTIBÁÑEZ AND T. PINTO. *Instituto de Psicología e Instituto de Fisiología. Universidad de Chile.*

Olfactory discrimination was tested in 5 adult male Macacus Rhesus. In 2 of them the test was performed after surgical removal of the ventral region of the temporal lobe (including the allocortex, the subcortical nuclei and the ventral region of the neocortex). In the other 3 the test was performed after removal of the lateral neocortex (areas TA, TE and TG) (Bonin, G. and Bailey, P. The neocortex of Macaca mulatta. The University Illinois Press, 1947). Both groups presented a deficient olfactory discrimination after the operation and this deficiency was more marked in those animals with lesions of the lateral neocortex.

December 20th, 1955

Oxygen consumption and urinary nitrogen in rats treated with Thyroxine under restricted food intake. BY C. GRADO AND H. HOFFMANN. *Instituto de Fisiología. Universidad de Chile.*

Rats were maintained with restricted food intake (7 g), 15 days previously to thyroxine administration.

Thyroxine raises O_2 consumption and nitrogen excretion; but N/cal relation decreases when ad libitum diet is given to the animals. The effect of thyroxine on the metabolic rate is decreased under restricted food intake; as N excretion remains raised N/cal ratio tends to increase.

If diet is supplemented with carbohydrates (5 g), the metabolic response to thyroxine raises to normal; N excretion decreases and consequently N/cal ratio decreases. Vitamin B_1 administration (10 mg % diet) does not exhibit any special effect. Brewer's yeast administration (2.5 % diet) reduces N excretion significantly.

Non-protein-nitrogen blood index is increased during hyperthyroidism. No special variation could be detected following to the administration of hypercaloric diet, supporting the evidence of a diminished protein catabolism.

Survival is significantly greater in rats under restricted food intake (16 days ± 1.1) than in ad libitum fed rats (10 days ± 0.09).

Cortisone administration (0.1-0.3 mg/day) does not affect mortality rate of thyroxine treated rats maintained with restricted food intake. Cortisone (0.3 mg daily) increases O_2 consumption but decreases N excretion. However, there is a definite increase in blood N. P. N.

There is a negative correlation (— 0.97) between energetic balance and mortality rate.

Autoglycolysis in the blood of hypothyroid and adrenalectomized rats. By J. M. Calvo and V. Ubilla. *Instituto de Química Fisiológica y Patológica. Escuela de Medicina. Universidad de Chile.*

Effects of cortisone on the production of isoagglutinins. By G. Hoecker and N. Kalies. *Instituto de Biología. Cátedra de Biología. Escuela de Medicina. Universidad de Chile. Jackson Memorial Laboratory, Ber Harbor, Maine.*

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Production of para-aminobenzoic acid by kidney slices of normal and hyperthyroid rats. By J. M. Calvo and W. Hülsen. *Instituto de Química Fisiológica y Patológica. Escuela de Medicina. Universidad de Chile.*